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Development of conceptuses during inanition in the rat and in the pig

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by

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TABLE OF CONTENTS

INTRODUCTION

Reproductive processes in most species evolved to guarantee survival of a maximum number of offspring to complete a life cycle. Some species accomplish this by production of numerous offspring into a competitive environment where few may survive. However, mammals provide an optimum environment to assure survival of the relatively few offspring they produce. From conception the female mammal provides a favorable environment, the uterus, for growth and development. Nourishment of the pregnant female during this time is important to in utero survival and development, and neonatal survival and development (Jones, 1976; Minkowski et al., 1974). After this period of in utero nourishment, young are expelled, and nurtured by the dam until they are independent of her.

Presently, our understanding of the role of each nutrient in maternal nutrition is limited. A critical examination is required to determine physiological and developmental changes in the mother and conceptus(es) during extreme nutritional states. The investigations in this report were designed to determine effects of prolonged nutrient deprivation, starvation, on embryo survival and growth, fetal survival and growth as well as physiological changes in the dam during this time.

REVIEW OF LITERATURE

Nutrition and Hormone Production

Results of past research indicate that adequate nutrition is essential for the action of hormones involved in regulating reproductive phenomena. For example, in 1917, Loeb noted that severe underfeeding produced atypical ovaries in the guinea-pig. Later Papanicolaou and Stockard (1920) fed guinea-pigs a diet consisting of only carrots and observed that ovulation was delayed. In another investigation, Barry (1920) starved rats for the first twenty days postmating to determine the effects of inanition on fetal development. Of 17 rats, only one was pregnant at Day 20. In an experiment with restricted dietary protein levels in rats, Guilbert and Goss (1932) observed that out of 14 positive matings in a group which received < 5% protein, only one animal maintained pregnancy. Nelson and Evans (1953) investigated the effects of dietary protein on pregnancy maintenance in rats, and found that those fed a protein-free diet from the day of breeding resulted in resorptions on the ninth or tenth day of gestation in 90-100%.
In a later study (1954) Nelson and Evans found that pregnancy rate was improved slightly (20-30%) by daily injections of 0.5 µg of estrone, while daily treatment with 4 mg of progesterone increased markedly the pregnancy rate (70-80%) in rats subjected to protein-free diets. However, a combination of the two steroids (4 mg progesterone and 1 µg estrone) maintained pregnancy in 100% of the protein-deficient animals.

Since the work of Nelson and Evans (1953; 1954) many investigations

have dealt with either the causes and/or prevention of nutritionally induced resorptions. For example, Kazancigil (1960) fasted rats for 5 days, (Days 3-8, postmating) and found that none were pregnant at autopsy on Day 14. But daily injections of progesterone and α-estradiol maintained pregnancy in another group of rats subjected to this same period of inanition. McClure (1961) determined that inanition in rats on Days 1-4, Days 6-10, and Days 12-16 after mating reduced the pregnancy rate to zero. McClure (1966) fasted mice for 48 hours during the fourth and fifth days after mating and pregnancy failure resulted in all mice. However, mice allowed to drink a 75% glucose solution during the same fasted period had a normal pregnancy rate. Restricted intake of a completely adequate diet also caused reproductive failure in mated rats as Berg (1965) found that only 8% of rats restricted to a 25% intake of a stock diet, Days 1-20, maintained pregnancy. Diets lacking one essential amino acid (valine or isoleucine) caused termination of pregnancy. However, this nutritionally induced resorption may not be a direct result of the missing amino acid since these rats lost body weight and pregnancy loss seemed to be associated with a decreased feed intake. Again in this study, embryonic wastage was corrected by daily injections of progesterone and estrone (Niiyama et al., 1970).

While it is well-established that exogenous progesterone and estrogen maintain pregnancy during times of inadequate nutrition, and atrophy of the reproductive tract associated with malnutrition is evidence of reduced steroid production (Leathem, 1966), the fault does not seem to be with the

steroid producing organ. In 1939, Pomerantz and Mulinos proposed that a pseudo-hypophysectomy condition resulted during severe underfeeding in rats, since ovaries and uteri of chronically starved rats weighed less and ovaries had fewer large follicles. These observations were similar to those in hypophysectomized animals. Also, reproductive tracts of malnourished animals remained responsive to exogenous gonadotropins (Mulinos and Pomerantz, 1941). Leathem (1966) stated that reduction of steroidogenesis by the ovary the first 10 days of pregnancy in malnourished rats suggests that prolactin release is inhibited. Some research has shown that exogenous prolactin injections maintained pregnancy in protein deficient rats (Leathem, 1966; Callard and Leathem, 1970). Also, Kalivas and Nelson (1966) demonstrated indirectly that prolactin maintained pregnancy in 64% of rats subjected to protein-free diets by administering reserpine, a tranquilizer known to release endogenous prolactin. Another method of elevating endogenous gonadotropins during dietary restriction was found to be exposure to constant illumination. When Piacsek and Meites (1967) subjected rats to a feed intake of half the control animals, ovarian weight decreased and interstitial and follicular tissues atrophied by 14-21 days later. In rats exposed to constant light for 10 days, growth of the interstitial and follicular tissues as well as a 50% increase in ovarian weight were observed. Exposure of mated rats to constant light (Days 1-19) maintained pregnancy in 50% of those fed a protein-deficient diet from the day of mating (Callard and Leathem, 1970).

Other investigations have determined the endogenous hormone levels

during protein deficiencies and restricted feeding of rats. Srebnik et al. (1961) measured the FSH and LH content of pituitaries from protein deprived rats and found that these pituitaries responded to ovariectomy with a marked rise in FSH and LH. Howland (1971) restricted feed consumption in rats to 50% of normal and noted a decrease in basal serum LH, but no difference in pituitary concentration indicating synthesis but little or no release of the gonadotropin. In pregnant rats, serum progesterone levels showed a dramatic decrease on Day 11 and remained low thereafter in those fed a protein-free diet from Day 1 (Giannina and Leathem, 1974). Further investigations of hormonal changes during nutritionally induced resorptions in the rat by Köhler et al. (1975) found that the level of maternal peripheral prolactin and progesterone in the serum decreased on Day 8. Recently, Hendricks and Bailey (1976) determined the maternal serum concentrations of prolactin and progesterone in rats allocated to three dietary levels of protein, (0%, 6%, and 18%) on Day 0 of gestation. Serum concentrations of progesterone and prolactin were lower in rats deprived of protein while the marginal dietary protein (6%) had no effect on hormone concentrations in the serum.

Evidence indicated that nutritionally induced pregnancy failure in the rat is the result of diminished release of luteotropic gonadotropins from the pituitary and not a fault of the placenta. In fact, short term injections, Days 5-9, of progesterone and estrone in rats given a protein-free diet maintained pregnancy until the placentae were established (Kinzey and Srebnik, 1963; Anderson et al., 1974). Luteotropic and

mammotropic activities of placentae from protein-deficient rats indicated that the endocrine function of the rat placenta, once established, was not hindered by the absence of dietary protein (Kinzey, 1968). The observation that dietary restrictions imposed after Day 10 have little effect on pregnancy rate (Berg, 1965; Köhler et al. 1975) supports this view.

Luteotropic support of the corpora lutea of pregnancy changes during pregnancy in the rat. Conversion of a corpus luteum of the cycle to a corpus luteum of pregnancy was dependent on prolactin secretion (Smith et al., 1976). However, after Day 7, LH was the primary luteotropic stimulus until Day 11 or 12 when rat chorionic mammotropin (placental luteotropin) supported the corpora lutea (Morishige and Rothchild, 1974). Rat chorionic mammotropin increased on Day 8 and by Day 12 reached maximal levels (Shiu et al. 1973). During the time when nutritionally induced resorption occurred, Days 8-11, the rat is dependent on progesterone from ovaries since the rat placenta is not capable of producing adequate progesterone to maintain pregnancy until after Day 16 (Csapo and Wiest, 1969).

Feto-placental Development

Gestational length varies among species. However, at the termination of a normal gestation in any species the neonate has developed to the extent that its survival in a new environment is assured. Occasionally neonates of a normal gestational length are retarded in their development at birth. This is termed intrauterine growth retardation (IUGR).

Natural occurrences of IUGR are "runts" in the farm animals and "small-for-gestational age" (SGA) infants in humans. The causes and consequences of IUGR in animal and human populations are of interest to scientists and physicians. Much of the information on IUGR was obtained from experimental models of IUGR in rats, guinea-pigs, sheep, pigs, and monkeys. Two major causes of IUGR extensively studied are maternal under-nutrition and reduced vascular supply to the placenta.

In general, maternal malnutrition is a reduction in total intake of a complete diet, or a reduction in protein or calorie intake. Restricted feed intake of half to one-third of normal in rats the last 6 days of pregnancy resulted in fetuses and placentae of normal weight (Campbell, et al., 1953). However, Campbell et al. (1953) found that a protein-free diet given during this time limited fetal and placental development. Later, Berg (1965) restricted rats to three levels (25%, 50%, and 75%) of feed intake during pregnancy and found that fetal weights varied inversely to the degree of restriction, and that feed restriction had the most detrimental effect on fetal weights when extended through the last half of gestation. Zamenhof et al. (1971) demonstrated that a protein-free diet fed to pregnant rats for five consecutive days the last half of gestation limited fetal and placental development. Naismith (1966) altered the protein level of diets from 13% to 6% and noted no change in fetal weight or chemical composition. However, dams fed 6% protein had a loss of protein from their carcasses. Zeman and Stanbrough (1969) examined fetal development in terms of RNA, DNA, and protein from dams given a 6% casein diet during gestation. Their data indicated that

maternal protein deficiency decreased cell numbers in fetuses the last 4 days of gestation. Young and Widdowson (1975) fed guinea-pigs a low energy diet (two-fifths of an adequate diet) or low protein diet (sucrose added) during the last half of gestation and noted that both diets limited fetal and placental weights. Rhesus monkeys fed an inadequate amount (0.5 g/kg body weight of protein during gestation delivered infants with lower birth weight (Kohrs, 1973). Also, as shown from data collected during the post war famine in Holland, maternal malnutrition limited birth weight and placental weight in humans (Smith, 1947; Stein and Susser, 1975).

Some investigations have examined the changes which occur early in gestation of the rats subjected to protein-free or low protein diets. Kenney (1975) and Hendricks and Bailey (1976) noted no effect on embryo weights by Day 10-12 when the dam was fed 5-6% protein. But in those rats given a protein-free diet, limited embryonic weight was apparent after Day 10. Köhler and Merker (1975) also investigated the growth (wet weights, DNA, and nitrogen) of embryos from rats subjected to a protein-free diet from Day 0 and found that by Day 7 there was a 58% growth retardation, and by Day 10 growth ceased completely.

Maintenance of pregnancy with progesterone and estrogen injections is often used to study fetal and placental development during severe dietary restrictions. For example, Kendall and Hays (1960) used steroid hormone treatments to investigate the effect of feeding pregnant rats sucrose throughout pregnancy and found that fetal weight after feeding sucrose to the dam for 20 days was 56% of normal. Also, Anderson et al. (1974) used

daily injections of progesterone and estrone to maintain pregnancy in rats fed sucrose, protein-free or casein diets. Again fetal weights were limited to about 50% of a normal Day 20 fetal weight during these dietary restrictions. Anderson (1975) subjected pigs to prolonged inanition of 45 days and found that by Day 34 the nitrogen (protein) content of embryos and placentae from these starved dams could be maintained at a level similar to those in animals on a full diet when exogenous progesterone and estradiol benzoate were supplied beginning Day 22. Endo et al., (1974) determined that at term fetuses from dams fed a protein-free diet maintained pregnant with progesterone and estrogen injections were limited to 52% of normal weight, and total protein (nitrogen), total DNA, and the protein DNA ratio (cell size) were all reduced in these fetuses. Although Morishige and Leathem (1972) used corticosterone to maintain pregnancy in dams fed a protein-free diet throughout pregnancy, they found fetal protein (nitrogen) and DNA reduced in fetuses from these deprived dams.

The pig has been the subject of many experiments in efforts to elucidate the effects of dietary energy and protein levels during gestation on embryonic and fetal development. For example, fetuses from pigs fed a high energy diet were larger at Day 60 and Day 90 when compared with fetuses of gilts on a low energy intake (Supnet and Eusebio, 1971). Self et al. (1960) reduced the daily feed intake of gilts during gestation to two-thirds and one-third of a full diet. Birth weight was reduced in those given one-third of a full diet and not affected by the two-thirds of a full diet restriction. Protein-free diets given to gilts

throughout pregnancy reduced birth weights of individual piglets while piglets from gilts given a protein-free diet from Day 24 to parturition had birth weights similar to those from gilts fed a control diet (Pond et al. 1969). Also, fetuses from dams subjected to 40 days of starvation had similar weights Days 70 and 110 of gestation while placental growth was limited during the inanition period (Parker et al. 1977).

Maternal malnutrition limits the growth of the conceptuses. However, maternal malnutrition differentially limits fetal organ development. For example, Buitrago et al. (1974) fed two groups of gilts either 2.2 (low) or 8.0 (high) M cal of digestible energy per day during pregnancy. Organs of the neonates were dissected and the weight of the liver, kidney, pancreas, and gastrocnemius were all limited in the low energy group to 25-40% of high energy values. While the adrenals and thyroid were limited only 10-20%, the brain weight of these neonates was not affected by the low energy diet of the dam. Atinmo et al. (1974) investigated the effects of an energy-or protein-restricted diet fed to gilts during gestation. Offspring from dams subjected to the protein restriction had reduced brain and liver weights at birth. However, brain DNA concentration (mg/g of fresh tissue) was similar in offspring from gilts fed an adequate diet, or an energy or protein restricted diet. In rats, Zeman and Stanbrough (1970) fed a low (6%) protein diet during pregnancy and observed that the weights and DNA content of the brain, liver, kidneys, heart, and thymus were all reduced in Day 20 fetuses as well as in neonates.

A protein-free diet fed 5 consecutive days the last half of gestation resulted in a significant decrease in fetal rat brain weights and brain DNA content (Zamenhof et al. 1971). In rats fed a protein-free diet throughout gestation, livers and brains of their fetuses had reduced total DNA and protein (nitrogen) but the protein-DNA ratio (cell size) of livers was normal while the cell size of brain cells was smaller than that from full-fed fetuses (Endo et al. 1974). Minkowski et al. (1974) has reviewed the changes which occur during IUGR in several species. Generally, the liver is more limited in development than the remainder of the body organs. In all species studied, this is a constant feature, that is the reduction of liver weight and total DNA content while there is conservation of brain weight and total DNA. In reference to other organs, the reduction of the maternal food intake produced IUGR of mainly the extraneural organs (Jones, 1976).

Several other factors also must be considered when examining IUGR. Nutrients supplied to the fetus from the mother are controlled by the blood supply to the placenta. For example, under favorable conditions during pregnancy, McLaren and Richie (1960) explained variations in fetal growth on the basis of the blood flow to which the placenta is subjected. In general, blood flow is greatest at the ovarian and cervical end of each uterine horn. Surgical alterations of placental blood flow reduced fetal growth rate (Jones, 1976). Hohenauer and Oh (1969) ligated a uterine artery Day 17 and produced IUGR of rat fetuses on Day 21 as evidenced by the weight and chemical composition of the fetuses. Joyce and Young (1974) reduced placental blood flow by hemorrhage in the pregnant

guinea-pig and noted that a 35% reduction in placental blood flow was accompanied by a 35% reduction in the transfer of amino nitrogen and glucose across the placenta. Under normal conditions, placental transfer of nutrients (glucose and α-aminoisobutyric acid) increased markedly the last days of pregnancy in the rat (Rosso, 1975a). Maternal undernutrition limited the transfer of nutrients (α-aminoisobutyric acid) to the fetus since placentae from malnourished dams assimilated α-aminoisobutyric acid from maternal circulation but they were unable to release it to the fetuses (Rosso, 1975b).

Fetuses from deprived dams exhibited altered lipid, carbohydrate and amino acid metabolism which are under some endogenous endocrine control (Jones, 1976). In rats, Shrader and Zeman (1973) demonstrated that growth hormone production was less in rat pups from protein-deficient (6% protein) dams. However, the regulation of fetal growth by fetal growth hormone is not clear. Fetuses of rats, mice, and rabbits decapitated *in utero* continued to grow (Jost and Picon, 1970). Experimental *in utero* ablation of the pituitary in lambs, and calves caused a considerable reduction in growth (Dawes, 1976). While in pigs, Atinmo *et al.* (1976) found that at week fifteen of gestation, fetuses from dams fed a protein restricted diet (0.5% protein) had lower body weights and plasma insulin levels than fetuses from control (18% protein) gilts. Insulin may be a major determinant of fetal growth (Dawes, 1976).

Maternal Metabolic Adaptations During Pregnancy

Adequately nourished pregnant animals assimilate nutrients for growth of a conceptus or conceptuses which may represent, in some instances, at term a significant proportion of the body weight of the dam. At the same time, the pregnant dam demonstrates a substantial increase in body weight during gestation. Hartwell (1927) reported an increase in maternal body weight in a majority of rats which completed a normal gestation. Abramson (1934) extensively investigated the body and organ weight changes that occur during pregnancy in the rat. He concluded that the body weight and liver weight showed the most marked change during pregnancy. During the first 13-15 days of pregnancy, rats fed ad libitum an adequate diet gained 45-55 g and most of this gain was attributed to maternal tissues since the uterus and conceptuses represented an insignificant weight increase (Campbell et al. 1953; Souders and Morgan, 1957; Naismith, 1966; and Kumaresan and Turner, 1968). The body weight gain during the last 6 days of pregnancy was mostly accounted for by the growth of the uterus and conceptuses (Campbell et al. 1953; Souders and Morgan, 1957). According to Zamenhof et al. (1971), after Day 15 the nutrient requirements of the conceptuses are no longer insignificant. Hytten and Thomson (1968) summarized the many physiological adjustments made during pregnancy and noted that the maternal body weight is increased even after discounting the products of conception, increased blood volume, and enlargement of the reproductive organs.

The nature of this increase in maternal body weight has been the

subject of many investigations. An early study by Poo et al. (1939) examined the body composition of pregnant rats and showed that a protein (nitrogen) increase in the liver and alimentary tract contributed most to anabolism during gestation in the rat. Newton (1952) reviewed metabolic changes during pregnancy and cited examples of nitrogen (protein) retention in many pregnant animals if adequately fed during pregnancy. As the body weight of the rat increased through pregnancy so did nitrogen (protein) retention (Beaton et al. 1954). Naismith (1966) found that during the first 14 days of pregnancy in the rat, both fat and protein (nitrogen) stores were augmented. Naismith (1973) conducted a nitrogen balance study and determined that pregnant rats used protein more efficiently as pregnancy advanced. In fact, nitrogen (protein) retained was more than double the amount in the conceptuses (Naismith, 1973; Naismith and Ritchie, 1973). During severe malnutrition in pregnancy the dam's carcass was the most important source of nitrogen (protein); (Niiyama et al. 1973) and utilization of her carcass was accomplished with little wastage (Newton, 1952; Berg, 1965).

Pregnant rats eat more food, gain more body weight at a faster rate (Kumaresan and Turner, 1968), and use food more efficiently (Newton, 1952; Berg, 1965; Naismith, 1973) than unmated animals of a similar age. Causes of these phenomena have been studied at different levels and by different methods. For example, Campbell et al. (1953) performed fectectomies in rats on Days 12-15, and demonstrated that the increase in maternal body weight was due to viable placentae acting through the ovaries or adrenals. Hervey and Hervey (1967) injected female rats with 5 mg of progesterone

daily and noted that these rats gained at the rate of 2 g per day while uninjected rats gained only 0.4 g per day. After injections for one month, the progesterone treated rats had increases in lean and fat tissue. Male rats did not respond to this progesterone treatment (Hervey and Hervey, 1967). Also, exogeneous progesterone 5 times the mean endogenous production level given to pregnant rats did not affect maternal weight gain or weight of the conceptuses (Bartholomeusz and Bruce, 1976). After the first week of pregnancy in the rat, nitrogen (protein) was retained by the reduction of fecal and urinary nitrogen excretion (Naismith and Fears, 1972b; Naismith and Ritchie, 1973). The results of Naismith and Fears (1972a) also implicated progesterone as the hormone responsible for this anabolism in early pregnancy, since the administration of progesterone to unmated rats reduced the plasma concentration of corticosterone, a catabolic hormone, reduced argininosuccinate synthetase of the liver and raised the plasma amino acid concentration. Naismith (1966) proposed that pregnancy in the rat is comprised of an anabolic phase, Days 0-14, and a catabolic phase, Days 15 to parturition. The anabolic phase attributed to the action of progesterone, and the catabolic phase attributed to the action of glucocorticoids from the feto-placental unit. Glucocorticoids given to adrenalectomized rats by Mayer et al. (1976) demonstrated catabolic effects by decreasing body weight, gastrocnemius weight, and muscle protease activity. Also, muscle intracellular concentrations of glucogenic amino acids, alanine aspartate and glutamate were reduced by glucocorticoids. Herrera et al. (1969) suggested that the pregnant rat possesses the capacity for

"accelerated starvation" the last third of gestation since muscle catabolism, gluconeogenesis and free fatty acids were activated more rapidly and to a greater degree in the fasted pregnant rat than the fasted nonpregnant rat. Radioactive pyruvate was incorporated into glucose at a greater rate, while serum glucose dropped dramatically during a 48 hr fast in late gestation (Herrera et al. 1969). Later, Knopp et al. (1970) demonstrated that adipose tissue from Day 19 pregnant rats had an increase in free fatty acids content. Thus, the pregnant animal appeared to be poised to mobilize fat when needed. Hypertriglyceridemia of pregnancy was related to exaggerated synthesis of lipid by the liver and was duplicated in nonpregnant rats by injections of estradiol benzoate and progesterone (Kalkhoff et al. 1972). Triglycerides were found to rise in the plasma of pregnant rats and removal from circulation appeared normal during most of gestation. Only in late gestation was lipid removal diminished which yielded hypertriglyceridemia (Knopp et al. 1975). Based on their research, Knopp et al. (1973) proposed two phases of adipose tissue metabolism as a maternal adaptation for fetal growth. In the rat, by Day 12 feed consumption was elevated as was plasma insulin. However, plasma free fatty acids were lower than in virgin controls and glucose conversion to fatty acids was higher. By Days 19-21 food intake remained elevated as did plasma insulin while plasma glucose declined. Use of labelled glucose determined that fatty acid formation declined in pregnant rats to one-third that of virgin controls while free fatty acids in serum increased. Thus, Knopp et al. (1973) concluded that ingested glucose was available

for the rapidly growing fetuses and previously stored lipids were mobilized to provide an alternate energy source to the dam.

PART I: DEVELOPMENT OF CONCEPTUSES DURING INANITION IN THE RAT

INTRODUCTION TO EXPERIMENT I

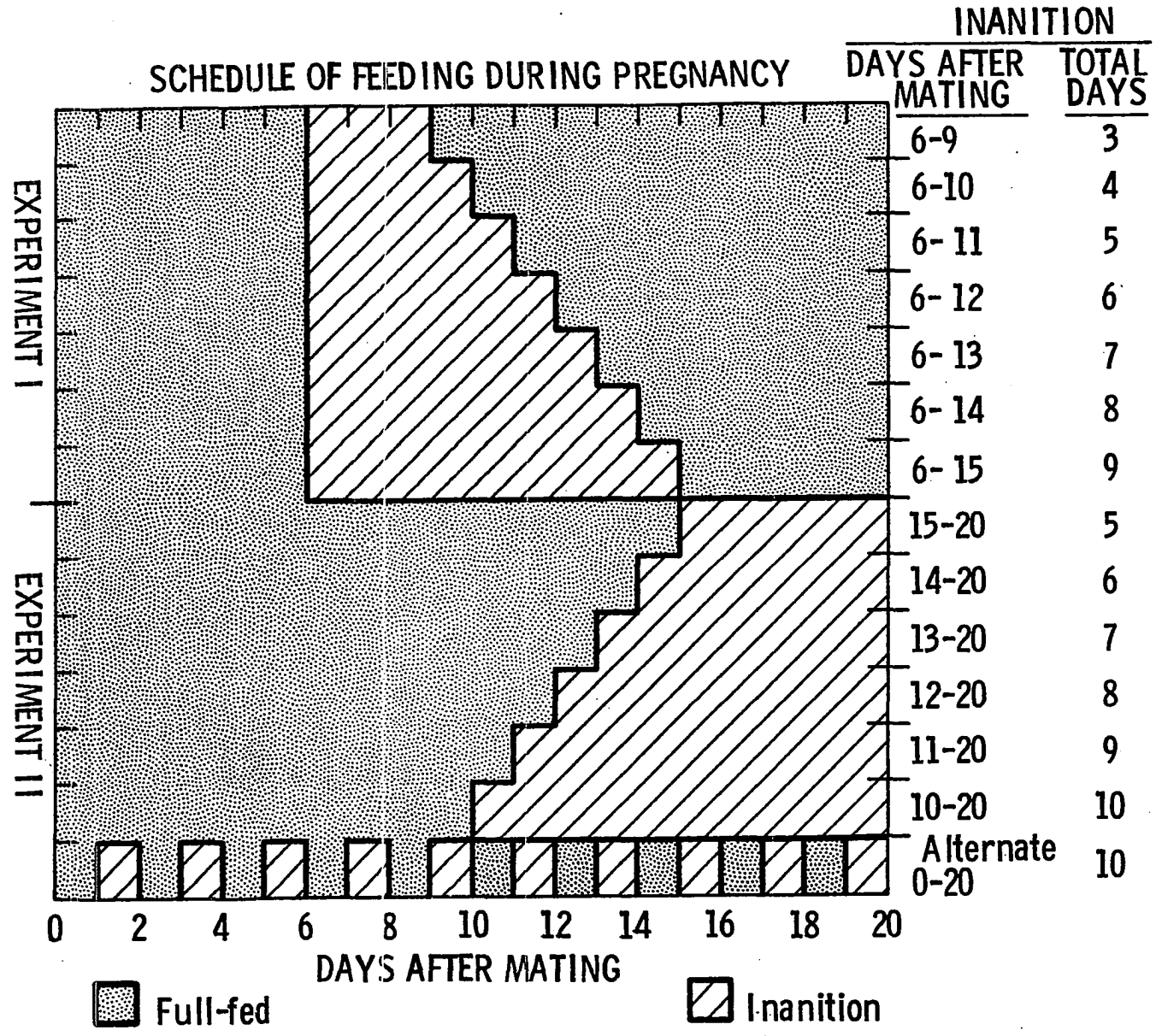
Protein deprivation after mating results in reproductive failure in rats (Anderson et al. 1974; Callard and Leathem, 1970; Nelson and Evans, 1953). However, when protein deprivation was initiated after implantation (Day 6) pregnancy failed in few animals (Köhler et al. 1975; Berg, 1965). Maternal stores of energy and protein are augmented the first two weeks of pregnancy in the rat during which time the uterus and conceptuses contribute little to the body weight of the dam (Knopp et al. 1973; Naismith, 1973). The purpose of this study was to subject pregnant rats to inanition beginning Day 6 and during augmentation of maternal stores to observe the effects on embryo survival and fetal development. Also changes in the dam during inanition and realimentation were observed.

MATERIALS AND METHODS FOR EXPERIMENT I

Albino rats (180-200 g), obtained from SASCO, Inc., (Omaha, Nebraska), were maintained on a 12-hour light and 12-hour dark schedule. Rats were fed ad libitum a commercially prepared diet (Teklad, Winfield, Iowa) with free access to water. Estrous cycles were followed by vaginal smears made daily between 8:00 a.m. and 10:00 a.m., and animals weighed approximately 250 g at the time of breeding. On the evening of the day of proestrus, a male was placed in the cage with the female and the following morning was considered Day 0 of gestation if sperm were present in the vaginal washing. Body weights were recorded daily.

On Day 6 after mating, rats were laparotomized to confirm pregnancy and determine the number of implantation sites. At this time, animals were assigned randomly to experimental groups. Control animals were fed ad libitum throughout pregnancy (Day 0 through Day 20) and rats in the treatment groups were maintained without feed beginning with the morning of Day 6. The pregnant animals remained without feed for 3, 4, 5, 6, 7, 8, or 9 days, thus on the morning of Day 9, 10, 11, 12, 13, 14, or 15 after mating, these animals were given feed ad libitum until autopsy on Day 20 (Figure 1). In addition, one group of 8 rats, subjected to inanition for 4 days (Days 6-10), was given daily subcutaneous injections of 5 mg of progesterone beginning the day before inanition (Day 5) and ending the day realimentation was initiated (Day 10). Another group of 8 rats, subjected to inanition Days 6-9, was laparotomized on Day 9 (day of realimentation) and each implantation site was injected with 0.1 ml of

Figure 1. Schedule of feeding during pregnancy for rats subjected to inanition in early pregnancy or rats subjected to inanition the latter half of pregnancy.


SCHEDULE OF FEEDING DURING PREGNANCY
INANITION
DAYS AFTER MATING
TOTAL DAYS
6-9 3
6-10 4
6-11 5
6-12 6
6-13 7
6-14 8
6-15 9
15-20 5
14-20 6
13-20 7
12-20 8
11-20 9
10-20 10
Alternate 0-20 10
EXPERIMENT I
EXPERIMENT II
0 2 4 6 8 10 12 14 16 18 20
DAYS AFTER MATING
Full-fed
Inanition

sterile 3% NaCl to assure pregnancy failure. During these periods of inanition, rats had free access to water.

On the afternoon of Day 20, rats were killed with chloroform, autopsied, and the following organs were weighed: uterus plus conceptuses, uterus, adrenals, ovaries, liver, and right gastrocnemius muscle. The number of viable fetuses, number of necrotic sites, fetal weights, and placental weights also were obtained.

Body composition was determined as described by Barnett and Widdowson (1971). Rats were weighed to the nearest 0.1 g and skinned. All adherent fat and muscle were removed from the skin and returned to the carcass. After weighing, the adrenals, ovaries, liver, and gastrocnemius were also returned to the carcass. Any blood lost during dissection was returned to the carcass. The carcass, skin with hair, and fetuses were dried at 96°C to constant weight, and water content was determined by difference. The skin, carcass, and fetuses were wrapped separately in filter paper and extracted with petroleum ether A (b.p. 40-60°C). The petroleum ether was removed and replaced until the extract became colorless. Lipid-extracted samples were again dried in an oven at 96°C. Lipid content was determined by the difference between the dry weight and the dry lipid-free weight. Dry lipid-free skins, carcasses, and fetuses were ground in a Wiley mill. Placentae were dried at 96°C and ground with a mortar and pestle. Nitrogen was determined in duplicate samples (100-200 mg) of ground skin, carcass, fetus, and placenta by macro-Kjeldhal method. Samples (1-2 g) were ashed to 700°C for 12 hours in a muffle furnace.

Data were analyzed by computing Student's t based on a weighted average of sample variances (Steel and Torrie, 1960).

RESULTS OF EXPERIMENT I

The effects of inanition on pregnancy rate (number of dams with viable fetuses on Day 20 divided by number of dams with implantation sites on Day 6) and embryonic survival rate are presented in Table 1. These data show that rats starved for 3 days (Days 6-9) maintained a pregnancy to Day 20. Few rats subjected to an inanition period longer than 4 days remained pregnant. Pregnancy rate of the group without feed for 4 days (Days 6-10) was 17%; overall pregnancy rate for groups in which inanition exceeded 3 days also was 17%. All eight rats injected with progesterone (5 mg/day) Days 5-10 and subjected to inanition Days 6-10 maintained a pregnancy to Day 20.

Animals which remained pregnant through the inanition intervals demonstrated a high embryonic survival rate similar ($P > 0.05$) to that in the ad libitum-fed controls. The combined embryonic survival rate for all rats subjected to inanition was 93 $\pm$ 2.1% and that of controls was 93 $\pm$ 2.1%. Rats injected with progesterone also had a high embryonic survival rate similar ($P > 0.05$) to that observed in full-fed animals (Table 1).

The body weight in ad libitum-fed pregnant rats increased 40 g from Day 0 to Day 14, whereas unmated ad libitum-fed animals gained only 6 g during 14 days. Body weight in full-fed mated controls increased abruptly (e.g., 69 g) from Day 14 to Day 20; unmated full-fed animals gained only 11 g during a similar 6 day period (Figure 2). Figure 3 indicates body weight changes in starved animals. Body weight loss was the greatest ($P < 0.01$) the first day of inanition and body weight decreased at a

Table 1. Pregnancy rate and embryonic survival in rats subjected to inanition beginning Day 6

Days of gestation dam subjected to inanition	Number days of inanition	Number dams with implantation sites on Day 6	Number dams with fetuses on Day 20	Pregnancy rate (%)	Viable fetuses per dam Day 20[a]	Embryonic survival rate (%)[a,b]
None	0	18	18	100	11 ± 0.5	93 ± 2
6-9	3	17	17	100	12 ± 0.5	95 ± 2
6-10	4	23	4	17	13 ± 1.0	96 ± 4
6-10 + Prog[c]	4	8	8	100	10 ± 1.0	89 ± 7
6-11	5	19	2	11	8 ± 5.0	81 ± 6
6-12	6	18	1	6	13	93
6-13	7	19	2	11	11 ± 1.0	79 ± 7
6-14	8	18	7	39	13 ± 0.8	95 ± 2
6-15	9	16	3	19	13 ± 0.4	97 ± 3

[a] Mean and S.E.

[b] Viable fetuses on Day 20 divided by the number of implantation sites Day 6.

[c] Progesterone, 5 mg/da, s.c., Days 5-10.

Figure 2. Body weight for ad libitum-fed rats (O) during 20 days of gestation and body weight of ad-libitum fed unmated rats (●) during a 20 day period

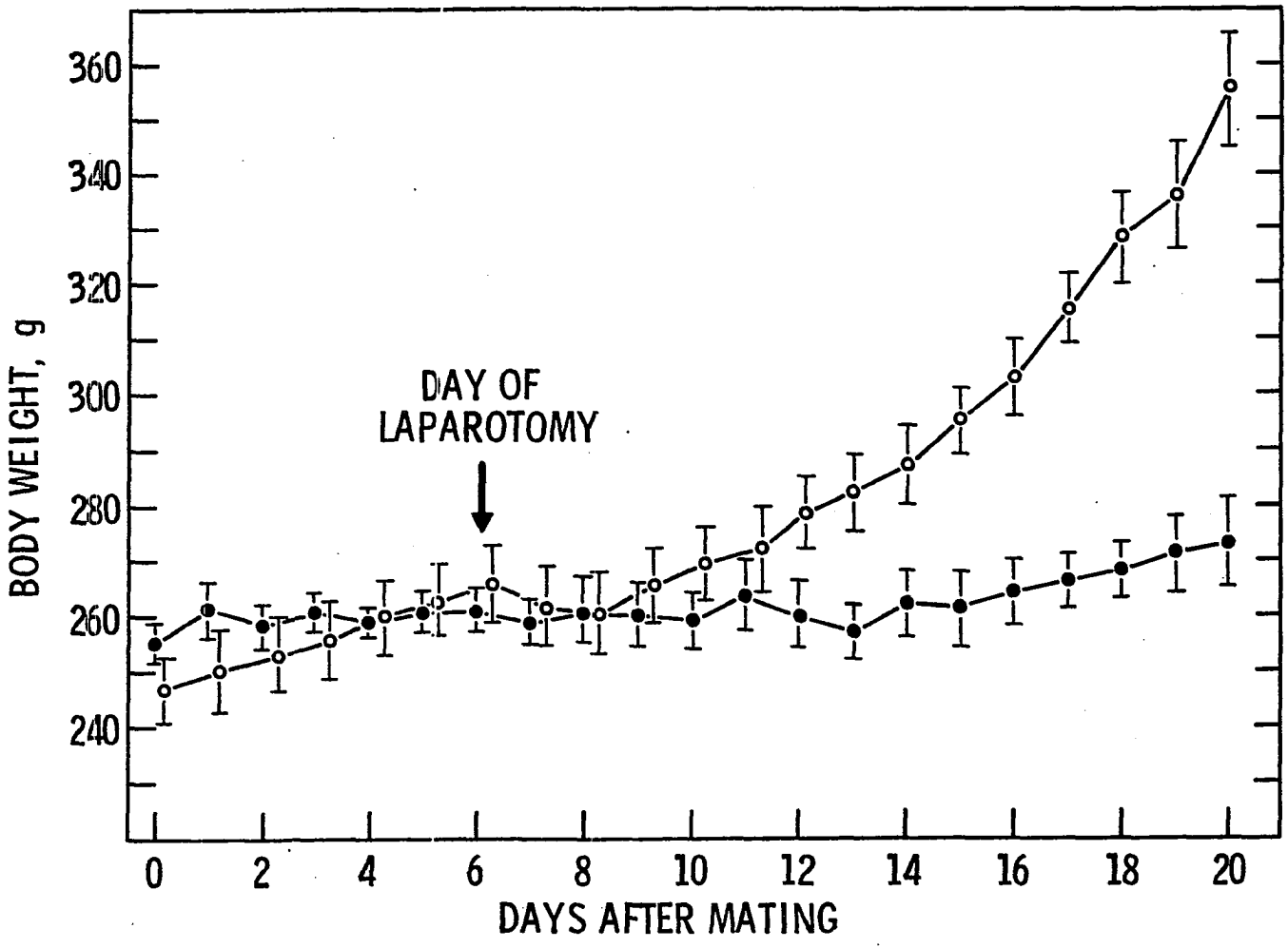
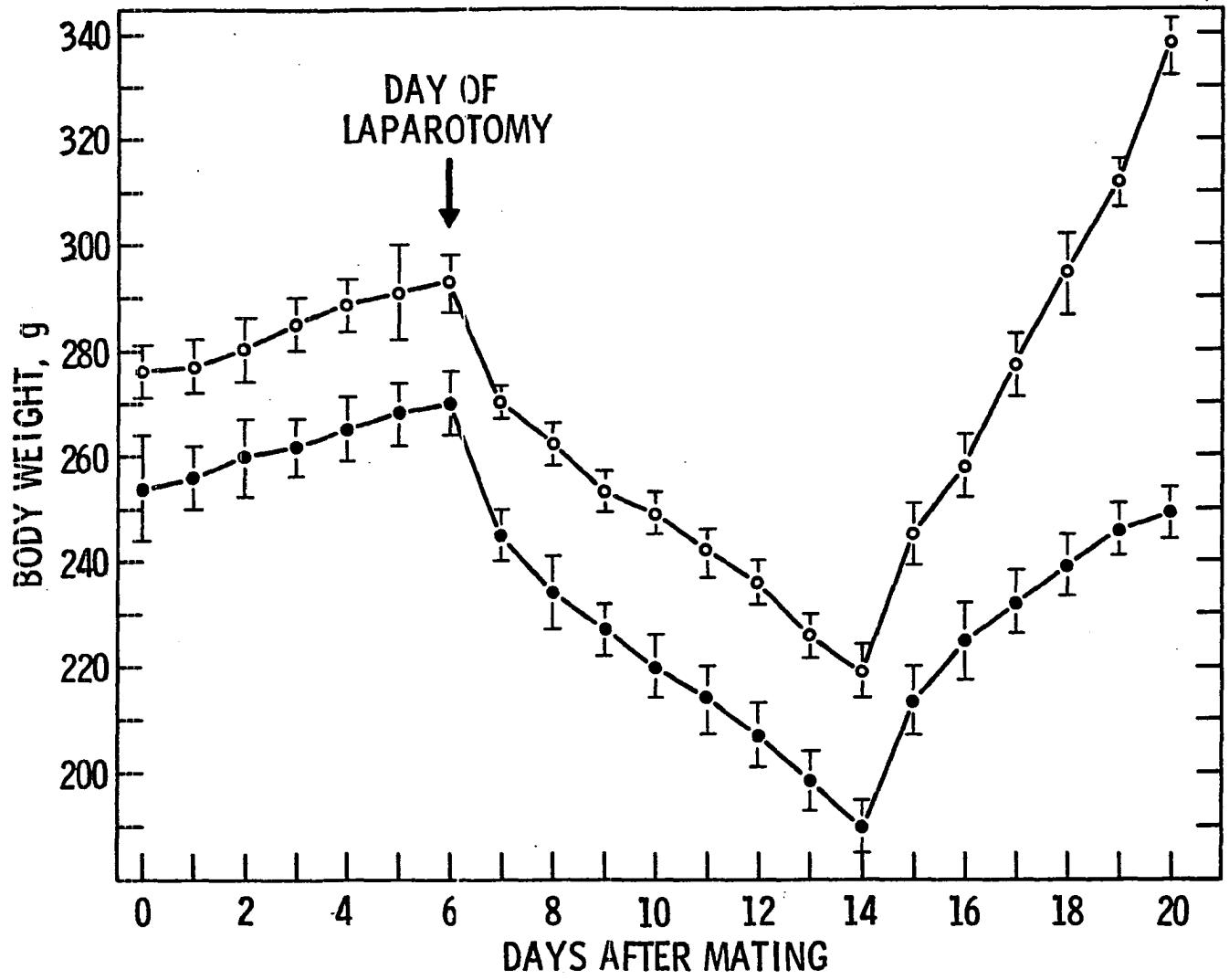

BODY WEIGHT, g
360
340
320
300
280
260
240
DAY OF
LAPAROTOMY
0
2
4
6
8
10
12
14
16
18
20
DAYS AFTER MATING

Figure 3. Body weight during 20 days after mating in rats which maintained pregnancy throughout inanition (O) and in those rats which pregnancy failed during inanition (●)


340
320
300
280
260
240
220
200
BODY WEIGHT, g
DAY OF
LAPAROTOMY
0
2
4
6
8
10
12
14
16
18
20
DAYS AFTER MATING

constant rate during the remaining days of inanition. Those animals maintaining pregnancy to the day of refeeding showed an abrupt increase in body weight from the day of refeeding to Day 20, and had a greater ($P < 0.01$) rate of gain Days 15 through 20 than ad libitum-fed pregnant rats (19 g/day vs. 12 g/day). Rats in which pregnancy failed during inanition rapidly increased in body weight for several days during realimentation, but by Day 20 body weight plateaued (Figure 3).

Body weight loss in rats subjected to inanition for 3 days (Days 6-9) was less ($P < 0.05$) than the weight loss of rats in all other groups subjected to inanition (Figure 4). However, the weight loss of pregnant rats and pregnancy-failed rats was similar ($P > 0.05$) for all groups. For example, in the group starved Days 6-14 (8 days) the 7 rats which maintained pregnancy lost 74 g while those not maintaining pregnancy lost 81 g (Figure 4).

From day of mating to Day 6, pregnant rats gained 18 $\pm$ 1 g of body weight while unmated rats gained 5 $\pm$ 1 g during a 6 day period. Figure 5 indicates that mated rats were capable of returning to their Day 6 (zero line of figure) body weight after a period of inanition. Pregnant rats not subjected to inanition gained 24 g maternal body weight (uterus plus conceptuses weight on Day 20 discounted) Days 6-20 of pregnancy, and unmated full-fed animals gained less ($P < 0.01$) during a similar period. Animals subjected to 3, 4, or 5 days of starvation (Days 6-9, 6-10, and 6-11), which maintained pregnancy through realimentation, showed a maternal weight increase above the Day 6 level

Figure 4. Body weight lost during inanition periods for those rats which maintained pregnancy and those in which pregnancy failed

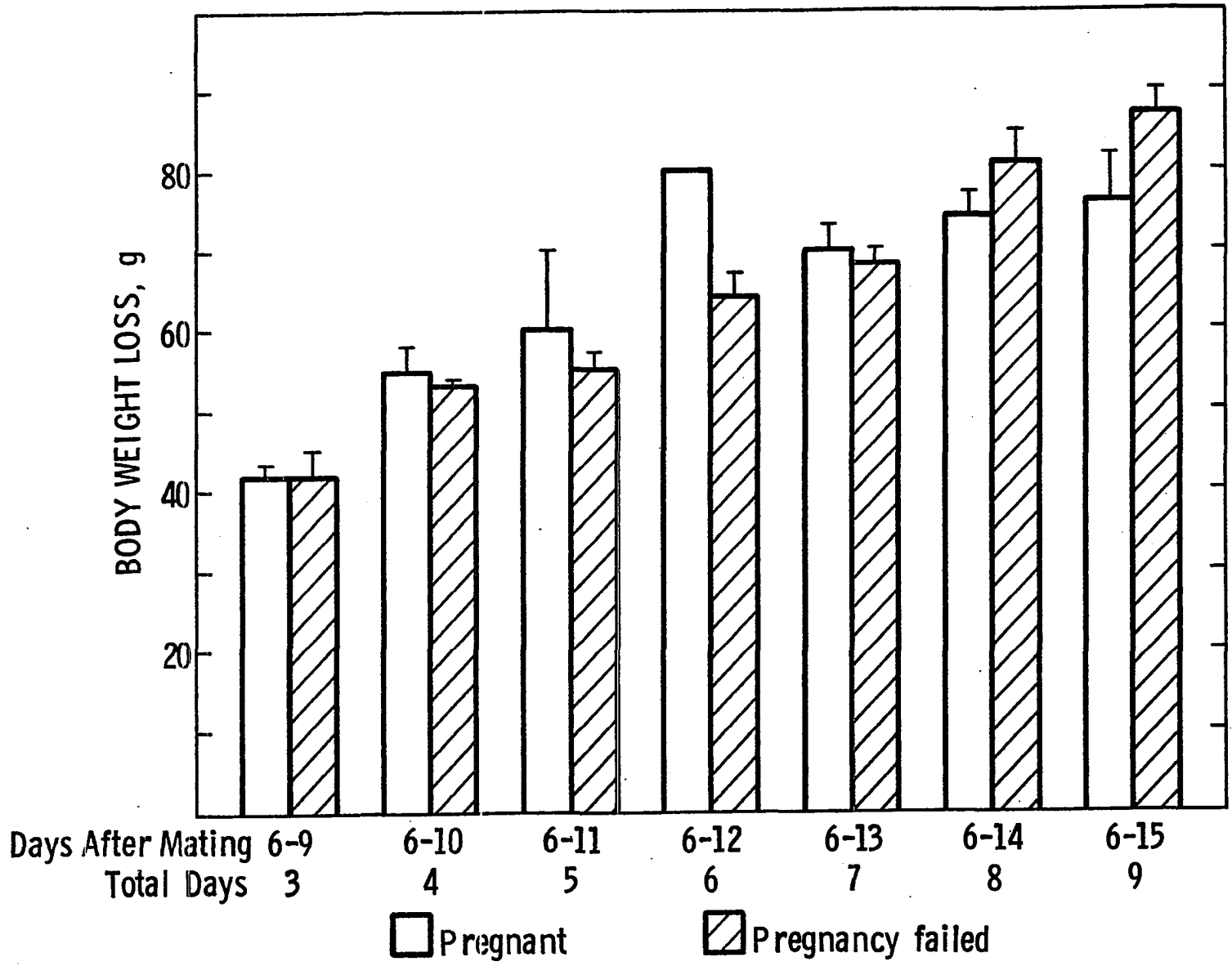
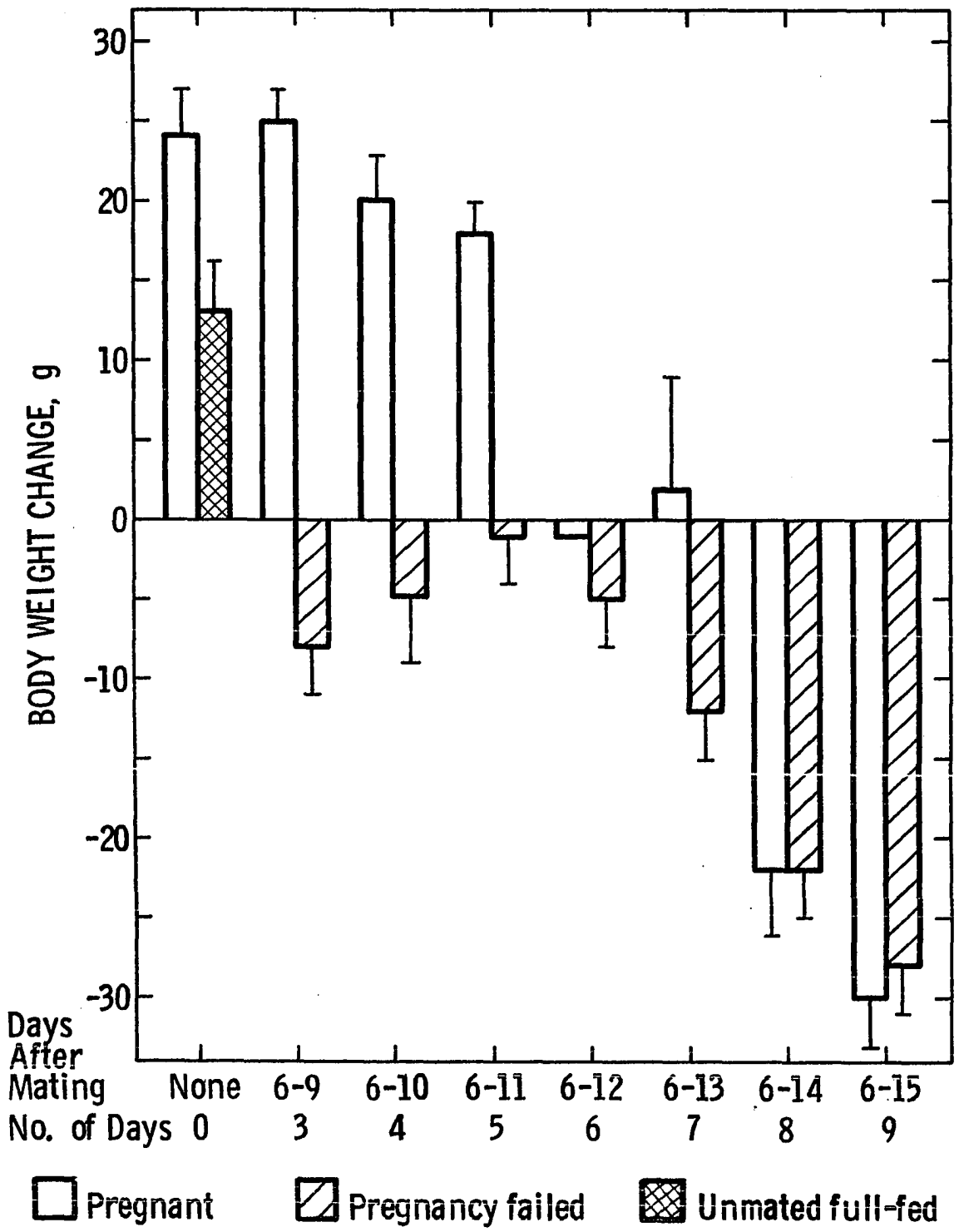

BODY WEIGHT LOSS, g
80
60
40
20
Days After Mating 6-9 6-10 6-11 6-12 6-13 6-14 6-15
Total Days 3 4 5 6 7 8 9
Pregnant
Pregnancy failed

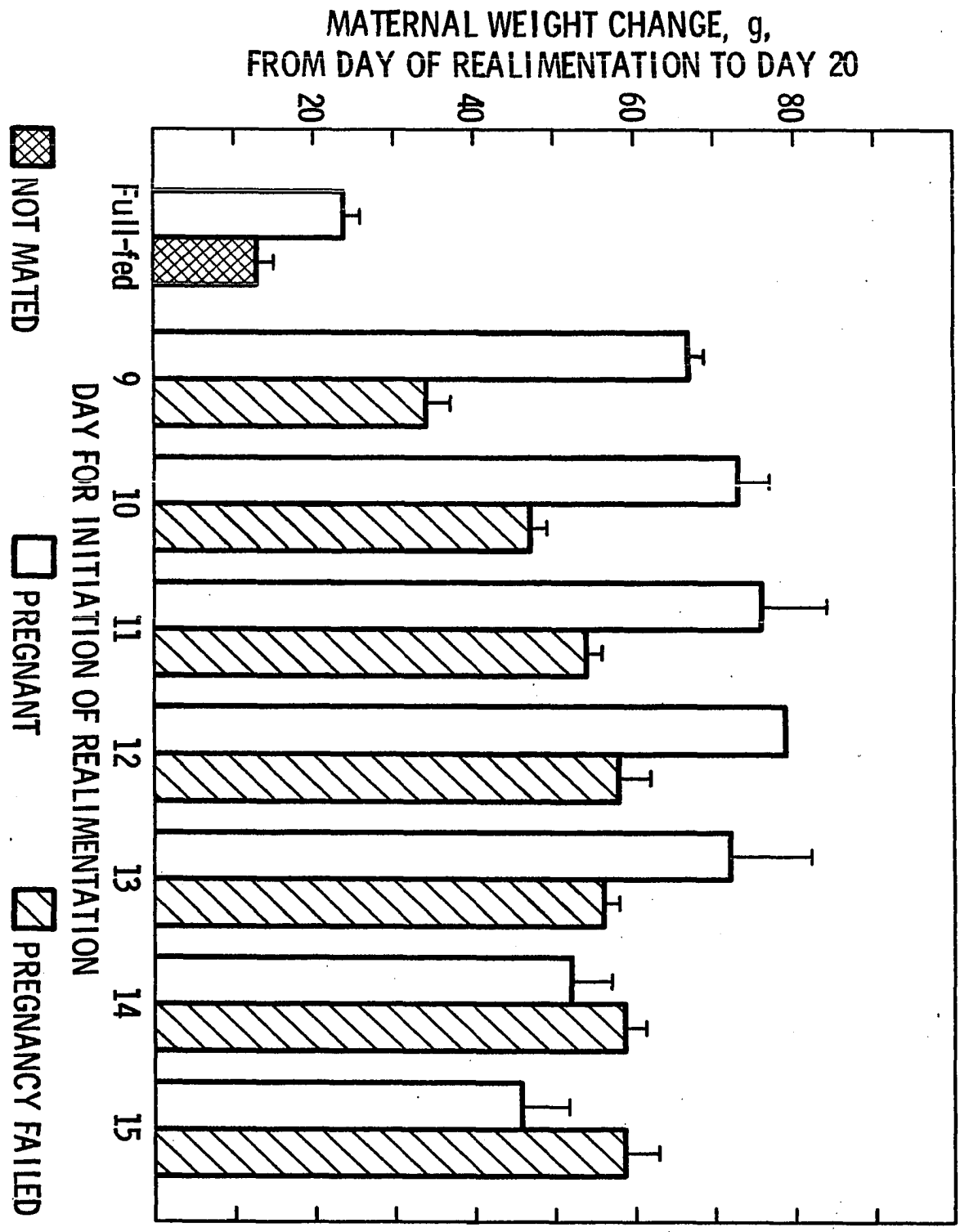
Figure 5. Body weight change, uterus plus conceptuses discounted, Days 6-20 in rats which maintained pregnancy and those in which pregnancy failed (zero point of figure is the Day 6 body weight)


30
20
10
0
-10
-20
-30
BODY WEIGHT CHANGE, g
Days After Mating
None
6-9
6-10
6-11
6-12
6-13
6-14
6-15
No. of Days
0
3
4
5
6
7
8
9
Pregnant
Pregnancy failed
Unmated full-fed

similar ($P > 0.05$) to ad libitum-fed pregnant animals (Figure 5). Rats maintained pregnant with progesterone injections during inanition Days 6-10 also had a maternal body weight increase similar ($P > 0.05$) to full-fed animals by Day 20. All animals in which pregnancy failed were unable to return to their Day 6 body weight. Even those rats subjected to 3 days (Days 6-9) of inanition and pregnancy termination induced on Day 9 with the NaCl injections were unable to return to their Day 6 weight (Figure 5). Dams starved 8 or 9 days (Days 6-14 or 6-15) showed a similar ($P > 0.05$) inability to return to their Day 6 weight regardless of whether pregnancy failed or was maintained (Figure 5). However, the pregnant females in these two groups also provided nutrients for the combined growth of the uterus and the conceptuses which amounted to 65 $\pm$ 3.7 g on Day 20.

Maternal body weight in full-fed pregnant rats increased 24 g during the last 11 days of gestation (Days 9-20; Figure 6). Rats subjected to inanition gained more ($P < 0.01$) body weight than this from the day of initiation of realimentation to Day 20 whether pregnancy failed or was maintained (Figure 6). However, those rats which maintained pregnancy gained more ($P < 0.001$) weight from the day of realimentation to Day 20 than did those in which pregnancy failed when realimentation was initiated on Day 9, 10, 11, 12, or 13. When realimentation was initiated on Day 14 or 15, pregnant and pregnancy-failed rats gained similar ($P > 0.05$) quantities of maternal body weight by Day 20. In all instances, pregnant animals provided for uterus and conceptuses growth not accounted for in the weight gain presented in Figure 6.

Figure 6. Maternal weight change (change minus Day 20 uterus plus conceptuses weight) during realimentation from day for initiation of realimentation to Day 20 after mating (zero point is body weight on day of initiation of realimentation)


MATERNAL WEIGHT CHANGE, g,
FROM DAY OF REALIMENTATION TO DAY 20
20
40
60
80
Full-fed
9
10
11
12
13
14
15
DAY FOR INITIATION OF REALIMENTATION
NOT MATED
PREGNANT
PREGNANCY FAILED

Those rats which remained pregnant throughout the inanition periods of 3-5 days showed little ($P < 0.05$) increase in maternal weight Days 15-20 when given 9 to 11 days of realimentation (Figure 7). This was similar ($P > 0.05$) to dams fed ad libitum (Figure 7). Unmated rats gained ($P < 0.01$) body weight during a 5-day period, and those pregnant and pregnancy-failed rats of longer inanition periods showed large body weight gains during Days 15-20.

Pregnant rats had heavier ($P < 0.05$) gastrocnemius muscles. However, the gastrocnemius weight in proportion to body weight was less ($P < 0.001$) in pregnant animals than nonpregnant animals 20 days after mating (Table 2). Ad libitum-fed pregnant rats had a gastrocnemius that was 4.7 mg/g body weight as compared with 6.1 mg/g body weight in full-fed unmated rats. The combined gastrocnemius weight for rats remaining pregnant after 4-9 days of inanition was 4.7 $\pm$ 0.1 mg/g of body weight, and similar ($P > 0.05$) to pregnant animals given ad libitum feeding throughout pregnancy. While the gastrocnemius for all rats in which the pregnancy failed was 5.8 $\pm$ 0.1 mg/g body weight; larger ($P < 0.01$) than in pregnant animals but similar ($P > 0.05$) to that in ad libitum-fed unmated females (Table 2).

Pregnant rats had heavier ($P < 0.001$) livers. Liver weight in proportion to body weight was similar ($P > 0.05$) in pregnant and pregnancy-failed rats by Day 20. Liver weight for all pregnant rats subjected to 4-9 days of inanition was 4.6 $\pm$ 0.1 g/100 g body weight and 4.4 $\pm$ 0.1 g/100 g body weight for all rats in which pregnancy failed (Table 2).

Figure 7. Maternal weight change (change minus Day 20 uterus plus conceptuses weight) Days 15 through 20 after mating (zero point is the Day 15 body weight)

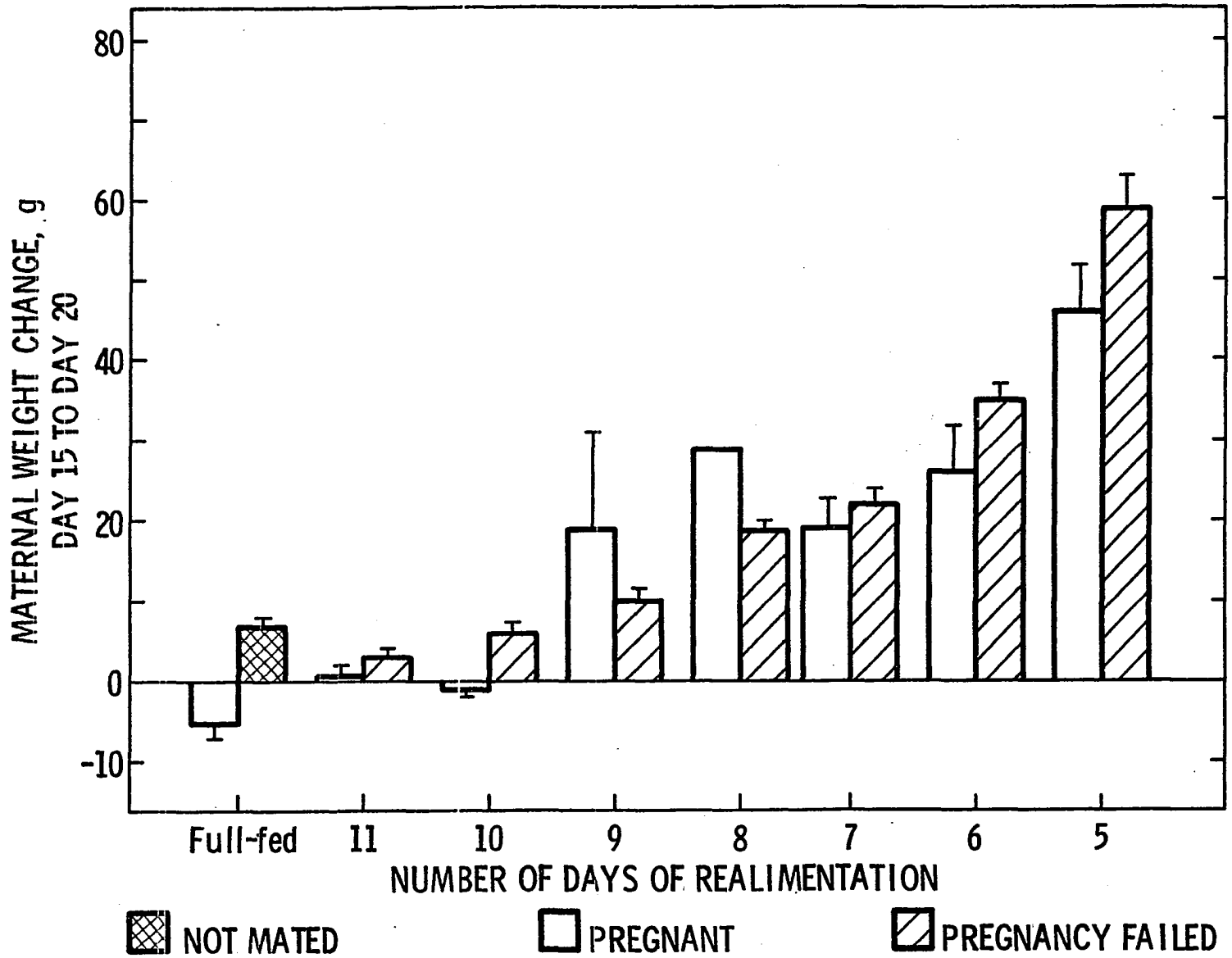

MATERNAL WEIGHT CHANGE, g
DAY 15 TO DAY 20
80
60
40
20
0
-10
Full-fed
11
10
9
8
7
6
5
NUMBER OF DAYS OF REALIMENTATION
NOT MATED
PREGNANT
PREGNANCY FAILED

Table 2. Weight of gastrocnemius, liver, ovaries, and adrenals of pregnant and pregnancy-failed dams

Days of gestation dam subjected to inanition	Number days of inanition	Gastrocnemius wt.[a]		Liver wt.[a]		Ovaries wt.[a]		Adrenals wt.[a]	
		g	mg/g body wt	g	g/100 g body wt	mg	mg/100 g body wt	mg	mg/100 g body wt
Pregnant									
None	0	1.67 ± 0.05	4.7 ± 0.1	15.2 ± 0.4	4.4 ± 0.2	103 ± 5	29 + 1.3	66 ± 3	19 ± 0.7
6-9	3	1.56 ± 0.04	4.3 ± 0.2	15.3 ± 0.7	4.2 ± 0.1	103 ± 5	28 + 1.1	71 ± 3	20 ± 0.7
6-10	4	1.94 ± 0.23	5.2 ± 0.4	16.2 ± 0.5	4.4 ± 0.1	114 ± 5	30 ± 1.0	67 ± 2	21 ± 1.0
6-11	5	1.74 ± 0.12	4.7 ± 0.1	16.6 ± 0.6	4.5 ± 0.3	124 ± 9	34 ± 1.0	67 ± 7	18 ± 0.3
6-12	6	1.47	3.9	18.2	4.9	128	34	72	19
6-13	7	1.54 ± 0.02	4.8 ± 0.1	15.1 ± 0.4	4.7 ± 0.3	118 ± 3	37 ± 0.5	59 ± 2	18 ± 0.0
6-14	8	1.52 ± 0.06	4.5 ± 0.4	15.3 ± 0.7	4.5 ± 0.2	104 ± 4	31 ± 1.4	72 ±3	21 ± 1.0
6-15	9	1.48 ± 0.07	4.8 ± 0.1	15.6 ± 0.4	4.7 ± 0.2	105 ± 7	32 ± 2.6	61 ± 7	18 ± 2.2
6-10 + Prog[b]	4	1.43 ± 0.04	4.4 ± 0.1	15.6 ± 0.4	4.4 ± 0.1	92 ± 6	30 ± 2.6	62 ± 3	19 ± 0.8
Pregnancy-failed									
None[c]	0	1.72 ± 0.04	6.1 ± 0.1	11.4 ± 0.5	4.0 ± 0.1	73 ± 7	22 ± 1.8	64 ± 4	22 ± 0.8
6-9[d]	3	1.73 ± 0.05	6.3 ± 0.2	11.2 ± 0.4	4.1 ± 0.2	90 ± 5	33 ± 1.8	62 ± 3	23 + 1.3
6-10	4	1.36 ± 0.04	5.3 ± 0.2	10.9 ± 0.2	4.2 ± 0.1	76 ± 4	23 + 1.1	79 ± 7	26 ± 1.0
6-11	5	1.51 ± 0.05	5.9 ± 0.2	11.0 ± 0.3	4.3 ± 0.1	77 ± 3	30 ± 1.1	63 ± 2	25 ± 0.8
6-12	6	1.51 ± 0.03	6.1 ± 0.1	10.7 ± 0.3	4.3 ± 0.1	67 ± 3	29 ± 2.6	59 ± 2	24 ± 0.8
6-13	7	1.49 ± 0.05	5.9 ± 0.1	11.0 ± 0.3	4.3 ± 0.1	61 ± 3	24 ± 1.0	53 ± 3	21 ± 1.1
6-14	8	1.37 ± 0.04	5.5 ± 0.0	11.1 ± 0.6	4.4 ± 0.2	65 ± 3	27 ± 1.2	59 ± 3	24 ± 1.1
6-15	9	1.48 ± 0.04	6.0 ± 0.1	11.7 ± 0.6	4.8 ± 0.2	54 ± 4	23 ± 1.8	56 ± 1	23 ± 0.5

[a] Mean and S.E.

[b] Progesterone, 5 mg/da, s.c., Days 5-10.

[c] Unmated.

[d] Sterile 3% NaCl injected into each implantation site Day 9.

Ovaries of pregnant animals were heavier ($P < 0.01$) than nonpregnant or pregnancy-failed rats (Table 2). Rats maintaining pregnancy through the longer periods of inanition (7-9 days) had ovaries similar ($P > 0.05$) in weight to full-fed pregnant animals on Day 20. Ovarian weights in pregnancy-failed rats decreased ($P < 0.05$) when the period of inanition exceeded 6 days (Table 2). Rats subjected to 5 to 9 days of inanition and remaining pregnant had similar ($P > 0.05$) ovarian weights per unit of body compared to full-fed pregnant rats by Day 20 (32 vs. 29 mg/100 g body weight). Ovarian weights per unit of body weight was less ($P < 0.01$) in those rats which pregnancy failed during 7-9 days of inanition. In these rats given progesterone to maintain pregnancy ovarian weights in proportion to body weight remained similar ($P > 0.05$) to ad libitum-fed pregnant animals (Table 2).

Rats maintaining pregnancy throughout inanition had larger ($P < 0.05$) adrenals at Day 20 than those in which pregnancy failed (Table 2). Adrenal weight in those rats remaining pregnant was 70 $\pm$ 1.8 mg and 60 $\pm$ 1.0 mg in rats which pregnancy failed. However, when adrenal weight was expressed on 100 g body weight basis, pregnant animals displayed less ($P < 0.01$) adrenal weight (19 $\pm$ 0.4 vs. 24 $\pm$ 0.4 mg/100 g body weight).

Starvation for a period longer than 6 days (groups 6-13 through 6-15) resulted in Day 20 fetal weights which were less ($P < 0.01$) than fetuses from ad libitum-fed animals or of those from dams subjected to shorter periods of inanition. The combined mean fetal weight for rats subjected to 3, 4, 5, and 6 days of inanition was 4.0 $\pm$ 0.2, similar

Table 3. Fetal and placental development on Day 20 of dams subjected to inanition beginning Day 6 after mating

Days of gestation dam subjected to inanition	Number days of inanition	Fetus wt, g[a]	Placenta wt, mg[a]	mg of placenta per g of fetus[a]	Uterus plus conceptuses wt, g[a]
None	0	4.0 ± 0.1	772 ± 21	196 ± 5	66.3 ± 2.9
6-9	3	3.8 ± 0.1	775 ± 9	203 ± 5	67.7 ± 3.5
6-10	4	4.0 ± 0.1	800 ± 36	198 ± 11	75.5 ± 7.3
6-10 + Prog[b]	4	4.1 ± 0.2	760± 36	189 ± 9	62.3 ± 7.7
6-11	5	3.8 ± 0.7	806± 84	215 ± 18	50.6 ±32.2
6-12	6	4.3	862	202	76.8
6-13	7	2.9 ± 0.2	633 ± 19	216 ± 21	51.5 ± 2.2
6-14	8	3.6 ± 0.1	683 ± 24	198 ± 9	66.9 ± 3.5
6-15	9	3.3 ± 0.1	671 ± 43	205 ± 11	60.5 ± 2.9

[a]Mean and S.E.

[b]Progesterone, 5 mg/day s.c., Days 5-10.

($P > 0.05$) to fetus from full-fed dams (Table 3). The fetal weight for animals starved 7, 8, or 9 days was limited ($P < 0.01$) to 3.3 ± 0.1 g. Placental growth was limited ($P < 0.01$) in animals starved 7, 8, or 9 days. Placental growth was similar ($P > 0.05$) in rats subjected to 3, 4, 5, and 6 days of inanition, and full-fed rats (Table 3). The ratio of placental tissue to fetal tissue was similar ($P > 0.05$) in those dams subjected to inanition and those full-fed. Despite the differences in fetal and placental development, the uterus plus conceptuses weight was 62.7 ± 2.7 in rats starved 7, 8, or 9 days, similar ($P > 0.05$) to that of rats subjected to shorter inanition periods and ad libitum-fed dams.

Composition of the dams' carcass and skin, fetuses, and placentae was determined for 8 full-fed rats and 5 rats which maintained pregnancy through inanition of 7, 8, or 9 days (inanition Days 6-13, 6-14, 6-15). These data are presented in Table 4. Carcasses of rats subjected to inanition weighed less ($P < 0.01$) on Day 20. Maternal tissues of these starved dams demonstrated an increase ($P < 0.01$) in the percentage of water in the carcass and skin, and a decrease ($P < 0.05$) in the percentage of lipid. Skins and carcasses of dams had a similar ($P > 0.05$) proportion of protein (N x 6.25) and ash. The percentage of water in fetuses and placentae was the same ($P > 0.05$) in full-fed and starved rats. Fetal and placental protein percentages were similar ($P > 0.05$) as were placental and fetal ash percentages from starved and ad libitum fed dams (Table 4).

Table 4. Body composition expressed as a percent of wet weight of dams, fetuses and placentae on Day 20

	Wet wt. g[a]	Water %[a]	Lipid %[a]	Protein %[a]	Ash %[a]
Full-fed (8)[b]					
Dam					
Carcass	239 ± 10.8	67 ± 0.6	11 ± 0.8	16.5 ± 0.5	4.4 ± 0.15
Skin	37 ± 1.8	51 ± 0.6	15 ± 0.5	30.6 ± 1.2	6.1 ± 0.25
Fetuses	4 ± 0.1	88 ± 0.1	2 ± 0.5	9.0 ± 0.1	1.3 ± 0.03
Placentae	0.73 ± 0.02	84 ± 0.6	-	12.7 ± 0.6	0.8 ± 0.03
Inanition (5)[b]					
Dam					
Carcass	216 ± 5.9	70 ± 0.5	7 ± 0.7	16.2 ± 0.3	4.9 ± 0.16
Skin	34 ± 1.1	55 ± 1.0	10 ± 1.8	32.5 ± 1.4	6.9 ± 0.56
Fetuses	3 ± 0.1	88 ± 0.1	2 ± 0.6	9.1 ± 0.1	1.3 ± 0.02
Placentae	0.65 ± 0.03	85 ± 0.6	-	12.6 ± 0.5	0.1 ± 0.05

[a]Mean and S.E.

[b]Number of dams.

DISCUSSION OF EXPERIMENT I

In this investigation, pregnancy failed in 83% of the rats subjected to inanition for periods exceeding three days; beginning at Day 6 after mating. This phenomenon was unexpected since inanition was initiated after implantation (Day 6), and protein-free diets fed beginning Day 6 did not cause pregnancy failure (Köhler et al. 1975). Severe nutritional deficiencies such as protein-free diets and dietary restriction initiated the day of mating caused pregnancy termination on or before Days 10 through 12 (Köhler et al. 1975; Anderson et al. 1974; Nelson and Evans, 1953). Also, heavy lactation caused pregnancy termination during this time (Veomett and Daniel, 1971). Pregnancy failure in this investigation occurred between Day 9 and Day 10. Ovarian weights of rats subjected to the longer periods of inanition in which pregnancy failed, 8-9 days, was less than that of ad libitum-fed cycling rats (Table 2) suggesting reduced trophic support. Pomerantz and Mulinos (1939) proposed that in rats a pseudo-hypophysectomy condition resulted from inanition. Howland (1971) found that restricted diet in female rats resulted in low serum LH levels and high levels of the gonadotropin in the pituitary, indicating synthesis, but little release of the gonadotropin. Köhler et al. (1975) and Hendricks and Bailey (1976) found the serum prolactin reduced by Day 8 in pregnant rats given a protein-free diet from the day of mating, Day 0. In the rat, luteotropic support shifts from the anterior pituitary to the placenta the first half of gestation. Pregnant rats hypophysectomized early in pregnancy required exogenous

progesterone and estrone Days 5 through 9, until the placentae were established (Kinzey and Srebnik, 1963). Prolactin was necessary for sustained luteal progesterone secretion through Day 7 and LH stimulated the corpora lutea Days 8 through 11 (Morishige and Rothchild, 1974). Luteotropin from the placentae reached highest levels by Day 12 (Shiu et al. 1973). The data in this study suggests that like pregnancy losses due to protein-free diets (Hendricks and Bailey, 1976; Köhler et al. 1975; Anderson et al. 1974), inanition beginning Day 6 and lasting longer than three days reduced luteotropic support from the anterior pituitary before the placentae secreted adequate luteotropin to maintain luteal function. Daily injections of progesterone, Days 5-10, allowed the placentae to establish.

Rats in this investigation which maintained pregnancy through the inanition periods had embryonic survival rates similar to that observed in pregnant rats fed ad libitum. Berg (1965) indicated that pregnancy either failed or maintained with high embryonic survival rates in rats exposed to severe dietary restriction. Anderson (1975) found the same pattern in pigs subjected to inanition early in pregnancy.

Rats which maintained pregnancy through inanition, based on body weight change, demonstrated a superior ability to realimentate compared to that observed in rats in which pregnancy failed. During realimentation, pregnant rats provided nutrients for growth of a uterus plus conceptuses similar in weight to that from dams fed ad libitum throughout pregnancy. Pregnant rats starved 3, 4, or 5 days (Days 6-9, 6-10, and 6-11) gained 50-75 g maternal

body weight during the 11, 10, or 9 days of realimentation (Figure 6). As Figure 5 illustrates, these rats maintaining pregnancy showed a net increase (Days 6-20) in maternal body weight similar to ad libitum-fed dams. Nonpregnant (pregnancy-failed) rats of those starved 3, 4, or 5 days (Days 6-9, 6-10, and 6-11) gained less body weight during realimentation, and all rats in which pregnancy failed were unable to return to their Day 6 body weight. For example, those rats subjected to only 3 days of inanition (Days 6-9) and 11 days of realimentation were unable to return to their Day 6 body weight when pregnancy failed (Figure 6). At the time animals attained their Day 6 body weight, they were pregnant and gained more body weight during 6 days of ad libitum feeding (Day 0 to Day 6 after mating) than did unmated rats for a similar 6 days ad libitum-fed period. Progesterone may be involved in the efficient performance of pregnant rats. Hervey and Hervey (1967) found that nonpregnant female rats injected daily with 5 mg of progesterone demonstrated greater body weight increases than did an oil injected group during one month. Naismith (1973) implicated progesterone in efficient utilization of proteins.

When realimentation extended into the last trimester (Days 13-20), the period of rapid conceptuses growth, the dam gained body weight (Figure 7) at the expense of fetal and placental growth. The mean fetal and placental weights of dams starved Days 6-13, 6-14, and 6-15 were less than that of fetuses and placentae from dams fed ad libitum throughout Days 0 to 20 or than that of fetuses and placentae from dams of shorter periods of inanition. Dams fed ad libitum or dams

subjected to short periods of inanition lost or maintained maternal body weight during rapid conceptuses growth Days 15-20 (Figure 7). Campbell et al. (1953) made a similar observation in ad libitum-fed pregnant rats. Therefore, the conceptuses competed with the realimentating dam for available nutrients in the dam subjected to inanition Days 6-13, 6-14, and 6-15. A 75% dietary restriction the first 13 days of gestation showed a similar trend of fetal growth during unrestricted feeding of an adequate diet the last 6 days of gestation (Berg, 1965).

Fetuses and placentae from dams replenishing body stores after 7 to 9 days of starvation (Days 6-13, 6-14, and 6-15) had similar proportions of water, protein, and ash compared to fetuses and placentae from dams fed ad libitum. Carcasses of these dams replenishing body stores after inanition were lighter, but the proportion of protein expressed on a unit basis was similar to that of the ad libitum-fed dams (Table 4). The absolute quantities of water, protein, and ash were quite different. For example, the water in fetuses from starved dams was 2.82 $\pm$ 0.08 g while that in fetuses from ad libitum-fed dams was 3.43 $\pm$ 0.08 g of water. Fetuses from ad libitum-fed dams contained 351 $\pm$ 9 mg of protein while starved fetuses contained 291 $\pm$ 9 mg. Similarly, carcasses of starved dams contained 35 $\pm$ 1.0 g of protein and carcasses of ad libitum-fed dams contained 39 $\pm$ 1.8 g of protein. Carcass analysis of rats fed high-fat and low-fat diets were observed to have similar percentages of water and protein but expressed as actual quantities of water and protein the two diet groups were different (Frisch et al. 1977).

Pregnancy in the rat is comprised of an anabolic phase (Days 0-14) when maternal stores are augmented and a catabolic phase (Days 15-parturition) when maternal stores and ingested nutrients are made available for the rapidly growing conceptuses (Campbell et al. 1953; Naismith, 1966; Knopp et al. 1973). Based on body weight gained, Figure 5 illustrates that rats given ad libitum feeding throughout gestation, or subjected to 5 days or less of inanition, augmented body weight over 20 days even after restoring that lost during inanition. Rats subjected to inanition and maintaining pregnancy during the change from an anabolic phase to a catabolic phase (Days 12-14) did not restore body weight lost during inanition (Figure 6) and had conceptuses which were smaller on Day 20.

Pregnancy failure occurred rapidly in rats subject to inanition after implantation. Embryonic survival rates were high, however, in those few rats maintaining pregnancy throughout inanition. Realimentation of the pregnant dams indicated that they effectively assimilated and utilized nutrients for their benefit as well as the benefit of the developing conceptuses.

SUMMARY

A total of 164 rats was used in these experiments and all rats were verified pregnant on Day 6 after mating by laparotomy. These rats were subjected to inanition beginning Day 6 for 3, 4, 5, 6, 7, 8, or 9 days at which time rats were given feed ad libitum until Day 20 after mating. Pregnancy failed in 83% of rats subjected to 4 days or more of inanition beginning Day 6. Progesterone injections given daily Days 5-10 (5 mg/day) maintained pregnancy in 100% of rats subjected to inanition. Those rats which maintained pregnancy during inanition had embryonic survival rates similar to rats fed ad libitum. Those rats which maintained pregnancy and those rats in which pregnancy failed lost similar quantities of body weight during inanition. However, pregnant rats gained more body weight during realimentation. Rats which maintained pregnancy possessed adrenal glands and gastrocnemius muscles smaller in proportion to body weight than those rats in which pregnancy failed. Liver weight increased or decreased in proportion to body weight whether pregnancy failed or was maintained. Fetal and placental development was limited when realimentation occurred during the third trimester.

INTRODUCTION TO EXPERIMENT II

Pregnancy requires the synthesis of large quantities of protein during a relatively short time. A pregnant rat must provide substrates for protein synthesis from her diet or tissue stores to conceptuses, which at term, compared to other mammals, represents a large percentage of her body weight (McKeown et al. 1976). Conceptuses of the rat grow rapidly the last 5-6 days of gestation, and this growth accounts for most of the body weight increase observed in dams during this time (Zamenhof et al., 1971; Naismith, 1966; Souders and Morgan, 1957). During the first two weeks of gestation when conceptuses are an insignificant portion of the dams' weight, rats augment body stores as evidenced by body weight gains of 40-50 g by Day 14 (Naismith, 1973; Campbell et al. 1953). Many investigations have examined fetal development during feeding of restricted or protein free diets (Morishige and Leathem, 1972; Callard and Leathem, 1970; Zeman and Stanbrough, 1970; Naismith, 1966; Berg, 1965). Only Barry (1920) has subjected pregnant rats to prolonged inanition. This study examined the effects of prolonged inanition during rapid growth of the conceptuses on fetal survival, fetal and placental development, and the body of the dam.

MATERIALS AND METHODS FOR EXPERIMENT II

In this second experiment, which involved inanition during the last half of gestation, the rats were maintained and bred as previously described (refer to page 20). Animals were assigned randomly to inanition periods after laporotomy on Day 6, to confirm pregnancy and count implantation sites. Eight rats were allotted to inanition beginning Day 10, 11, 12, 13, 14, or 15 of gestation and continuing through Day 20, the day of autopsy (Figure 1, page 22). In addition, eight rats were assigned to a group which were fed every other day, Days 0-20 (Figure 1). Eight rats allotted for a control group were fed ad libitum the commercially prepared diet throughout gestation. On Day 20, rats were killed by decapitation, 5-8 ml of blood were collected and allowed to clot for 30 minutes at room temperature. After centrifuging in a refrigerated centrifuge at 3500 RPM for 20 minutes, the serum was removed and stored at -20°C. The weights of uterus plus conceptuses, liver, heart, kidneys, adrenals, ovaries, and right gastrocnemius were recorded. Fetuses and placentae were dissected from the uterus, weighed, frozen on dry ice, and stored at -20°C; the uterus also was weighed.

Sixteen unmated rats with body weights similar ($P > 0.05$) to pregnant animals were subjected to inanition, weighed daily, and then killed by decapitation. Eight of these rats were starved 5 days and the other eight were starved for 10 days. At autopsy, the weights of the liver, heart, kidneys, adrenals, uterus, and right gastrocnemius were recorded.

Two frozen fetuses from each litter were homogenized in cold

distilled water (50 ml/g fresh tissue) with a Waring blender. Duplicate 1 ml aliquots of this homogenate were used to determine the DNA content of the fetuses by the indole method of Ceriotti (1952) and protein by the Lowry et al. (1951) method.

Serum calcium, potassium, and sodium were determined by atomic absorption from a 0.1 ml sample of the Day 20 maternal serum. To these 0.1 ml samples of serum, 3.0 ml of 4% trichloroacetic acid containing 2500 ppm of strontium (strontium chloride) was added to precipitate serum proteins and overcome the depression of calcium absorption by phosphate. After centrifugation at 2500 RPM for 10 minutes, the supernatant was used to determine calcium and potassium by use of a Techtron atomic absorption spectrophotometer (Cary Instruments, Monrovia, California). The absorbance of calcium was determined at 4226.7 Å while the absorbance of potassium was determined at 7764.9 Å. A 25 µl sample of the supernatant was diluted with 4.0 ml of lithium diluent, 150 meq/l, (lithium nitrate) and the absorbance at a wavelength of 5890.0 Å determined. Absorbance for known concentrations of calcium, potassium, and sodium were determined and graphed (absorbance against concentration). Unknown concentrations of sample absorbances were determined from this graph. Total protein in the Day 20 serum was determined using the biuret method (Reinhold, 1953) on duplicate 0.1 ml samples. The glucose oxidase-peroxidase reaction (Worthington Glucostat Reagent) was used to measure the Day 20 serum glucose level. Day 20 maternal serum urea was determined by the reaction of urea and 2, 3-butanedione-2-oxime in the presence of thiosemicarbazide (Marsh et al., 1965) using a Technicon autoanalyzer

(Technicon method file N-1c). Alpha amino nitrogen in Day 20 serum was also determined using the autoanalyzer by the trinitrobenzene sulfonate method described by Palmer and Peters (1966). Maternal Day 20 serum fatty acids were measured by a method described by Smith (1975). Maternal carcass dry matter was found by drying carcasses in an oven at 96°C to a constant weight. The lipid content of carcasses from dams subjected to the longest inanition period (10 days) was determined as previously described (refer to page 23).

Data were analyzed by computing Student's t based on a weighted average of sample variances (Steel and Torrie, 1960).

RESULTS OF EXPERIMENT II

During Days 0-20 pregnant rats fed ad libitum the commercially prepared diet gained 93 ± 3 g (Table 5). Those rats subjected to inanition gained weight at the rate of 2.5 ± 0.2 g per day before inanition, which was similar ($P > 0.05$) to full-fed animals between Days 0-15. Therefore, at the time inanition was initiated, Days 10 through 15, pregnant rats gained 23 to 40 g (Table 5). The overall body weight change between Day 0 and Day 20 ranged from -4 g for those subjected to inanition Days 15-20 to -49 g for those starved Days 10-20 (Table 5). Animals starved every other day, Days 0-20 gained 73 g (Table 5). After discounting the uterus plus conceptuses weight, ad libitum-fed animals gained 32 g, Days 0-20, while rats subjected to inanition lost 46 to 91 g (Table 5). Rats subjected to inanition every other day (Days 0-20) had a Day 20 body weight similar ($P > 0.05$) to their Day 0 body weight when the Day 20 uterus plus conceptuses weight was discounted (Table 5). Although the weight of the uterus plus conceptuses decreased ($P < 0.01$) in rats subjected to inanition, the uterus plus conceptuses remained a similar ($P > 0.05$) proportion of the Day 20 body weight when inanition began Days 10 through 15 to Day 20 (Table 5). Also in these groups subjected to inanition, the uterus and conceptuses as proportion of Day 20 body weight was similar ($P > 0.05$) to rats on ad libitum feeding throughout gestation. The weight of the uterus including conceptuses in rats starved every other day through Days 0-20 was greater ($P < 0.05$) in proportion to body weight (Table 5).

Table 5. Body weight changes observed in the dam before inanition, after 5 to 10 days of inanition the last half of pregnancy, and the Day 20 uterus plus conceptuses weight

Day of gestation dam subjected to inanition	Number days of inanition	Weight changes, g[a]			Uterus plus conceptuses wt., g[a]	Uterus plus conceptuses as a proportion of Day 20 body wt.[a]
		Body before inanition	Body[b] through gestation	Maternal[c] through gestation		
None	0	34 ± 2[d]	+93 ± 3	+32 ± 2	61 ± 2	0.18 ± 0.01
15-20	5	38 ± 3	-4 + 2	-46 ± 2	42 ± 3	0.17 ± 0.01
14-20	6	40 ± 3	-9 ± 3	-54 ± 3	46 ± 2	0.18 ± 0.01
13-20	7	32 ± 2	-20 ± 2	-60 ± 2	41 ± 2	0.18 ± 0.01
12-20	8	29 ± 4	-35 ± 2	-74 ± 2	39 ± 1	0.17 ± 0.01
11-20	9	29 ± 2	-41 ± 4	-77 ± 3	36 ± 3	0.16 ± 0.01
10-20	10	23 ± 3	-49 ± 3	-91 ± 3	42 ± 2	0.17 ± 0.01
Alternate 0-20	10	-	+73 ± 5	+1 ± 4	72 ± 4	0.21 ± 0.01

[a]Mean and S.E.

[b]Weight change Days 0 through 20 including uterus plus conceptuses weight on Day 20.

[c]Weight change Days 0 through 20 discounting uterus plus conceptuses weight on Day 20.

[d]Gain from Day 1-15.

In the 56 dams subjected to starvation, the number of viable fetuses on Day 20 was similar ($P > 0.05$; Table 6). Starved dams and ad libitum-fed dams sustained development of a similar ($P > 0.05$) number of viable fetuses by Day 20 (Table 6). Fetal survival ($P > 0.05$) was similar (viable fetuses on Day 20 divided by implantation sites on Day 6). (Table 6) between all starved rats and similar to that observed in rats fed ad libitum (Table 6).

Five consecutive days of inanition (Days 15-20) limited ($P < 0.01$) fetal and placental growth by Day 20 (Table 7). Fetal and placental weight declined as the number of days without feed increased to 10 days (Days 10-20). Those rats subjected to inanition every other day (10 days total) through gestation had fetuses and placentae which were similar ($P > 0.05$) to animals fed ad libitum through Days 0-20. Fetuses from dams subjected to 9 or 10 consecutive days of inanition had more ($P < 0.05$) placenta weight per g of fetus (Table 7). Although both fetal and placental development was limited ($P < 0.01$) in dams subjected to consecutive days of starvation there was a tendency for the fetuses to be more restricted than the placentae (Figure 8). After 5 days (Days 15-20) of inanition, both the fetal and placental weight were reduced to about 75% of that observed in Day 20 fetuses and placentae from dams fed ad libitum. After 9-10 days of starvation (Days 11-20 and 10-20) fetal weight was limited to about 50% and placental weight was limited to about 65% of ad libitum-fed fetuses and placentae (Figure 8).

Protein content of fetuses from dams starved 5-10 consecutive days

Table 6. Pregnancy rate and fetal survival in rats subjected to inanition the latter half of pregnancy

Days of gestation dam subjected to inanition[a]	Number days of inanition	Number dams with implantation sites on Day 6	Number dams with fetuses on Day 20	Pregnancy rate %	Viable fetuses Day 20[a]	Fetal survival rate %[a,b]
None	0	8	8	100	10 ± 0.4	97 ± 2
15-20	5	8	8	100	9 ± 0.8	90 ± 3
14-20	6	8	8	100	11 ± 0.7	95 ± 2
13-20	7	8	8	100	10 ± 0.4	94 ± 3
12-20	8	8	8	100	10 ± 0.4	94 ± 3
11-20	9	8	8	100	11 ± 1.1	89 ± 3
10-20	10	8	8	100	12 ± 0.6	95 ± 2
Alternate 0-20	10	8	8	100	12 ± 0.7	95 ± 1

[a]All rats autopsied Day 20.

[b]Number viable fetuses on Day 20 divided by the implantation sites at Day 6.

Table 7. Development of conceptuses by Day 20 in dams subjected to an interval of inanition during the latter half of pregnancy

Days of gestation dam subjected to inanition	Number days of inanition	Mean number of fetuses per dam[a]	Mean wt.[a]		Mg of placenta per g of fetus[a]
			Fetus, g	Placenta, mg	
None	0	10 ± 0.4	4.3 ± 0.07	724 ± 27	167 ± 5
15-20	5	9 ± 0.8	3.2 ± 0.12	549 ± 15	171 ± 5
14-20	6	11 ± 0.7	2.9 ± 0.12	541 ± 17	185 ± 6
13-20	7	10 ± 0.4	2.7 ± 0.12	496 ± 20	184 ± 7
12-20	8	10 ± 0.4	2.8 ± 0.12	512 ± 20	185 ± 9
11-20	9	11 ± 1.1	2.0 ± 0.19	439 ± 27	239 ±14
10-20	10	12 ± 0.6	2.3 ± 0.56	498 ± 14	219 ± 9
Alternate 0-20	10	12 ± 0.7	4.2 ± 0.12	719 ± 26	173 ± 5

[a]Mean and S.E.

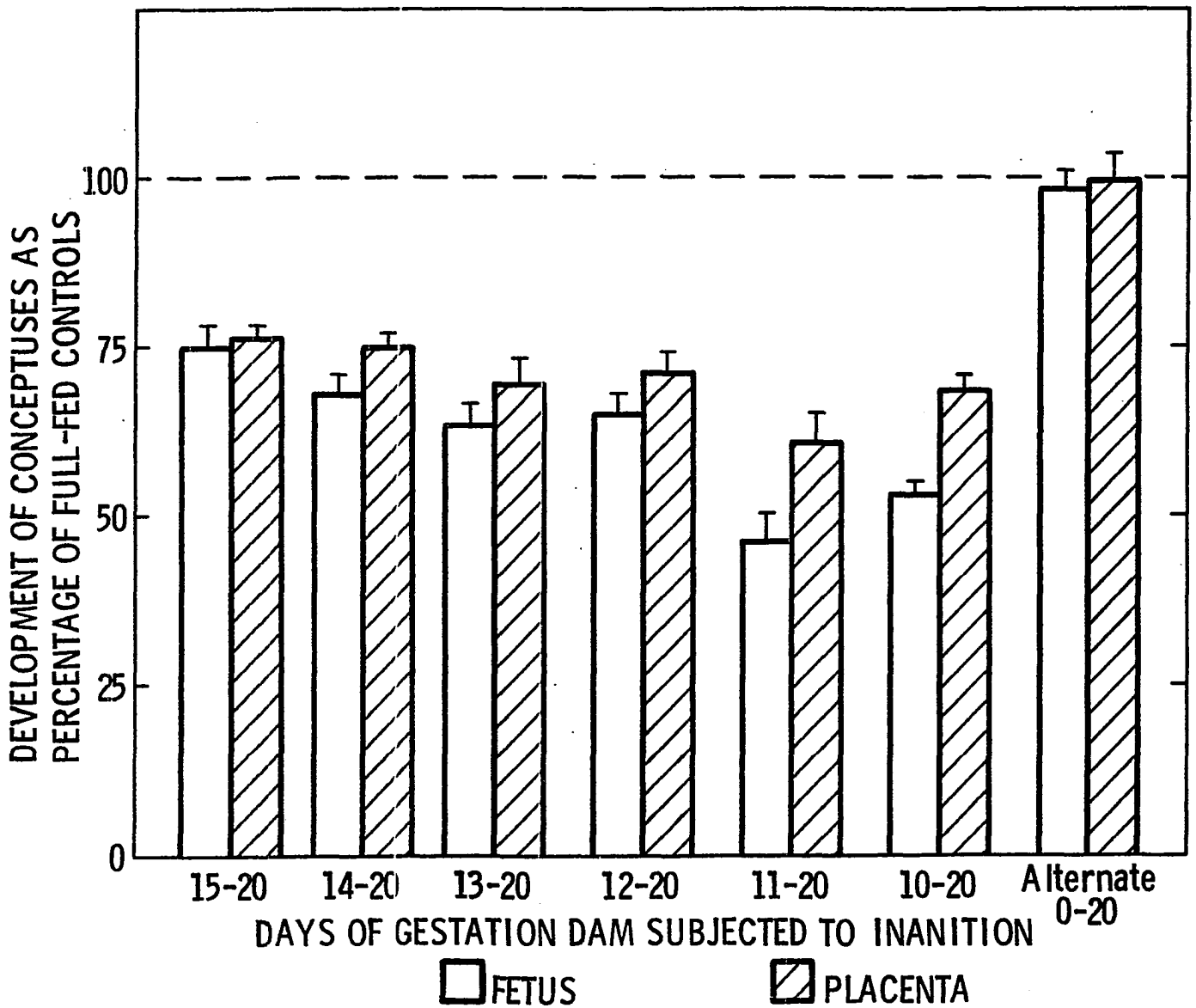
Table 8. Protein and DNA content of fetuses from dams subjected to inanition during the latter half of gestation

Days of gestation dams subjected to inanition	Number days of inanition	Protein[a]		DNA[a]	
		Total per Fetus, mg	mg per g of fetus	Total per fetus, mg	Protein DNA ratio
None	0	323 ± 37	76.6 ± 7.7	13.6 ± 0.6	25.0 ± 2.2
15-20	5	243 ± 31	75.6 ± 6.3	12.5 ± 0.3	19.2 ± 2.0
14-20	6	201 ± 17	70.6 ± 6.2	12.0 ± 0.5	16.3 ± 1.3
13-20	7	191 ± 16	68.8 ± 3.8	11.9 ± 0.5	16.2 ± 1.0
12-20	8	193 ± 19	74.3 ± 8.2	11.6 ± 0.6	16.3 ± 1.4
11-20	9	182 ± 27	68.6 ± 4.9	9.5 ± 0.9	15.7 ± 0.4
10-20	10	184 ± 20	76.0 ± 7.2	9.9 ± 0.6	17.3 ± 1.5

[a]Mean and S.E.

was reduced ($P < 0.01$). However, the quantity of protein on a unit basis was similar ($P > 0.05$) in fetuses from ad libitum-fed and starved dams (Table 8). Total DNA per fetus was reduced ($P < 0.05$) after 6 to 10 consecutive days starvation (Table 8). The protein-DNA ratio (mg protein divided by mg DNA) or cell size was reduced ($P < 0.01$) by inanition. Five days of inanition (Days 15-20) reduced ($P < 0.01$) fetal cell size (protein-DNA ratio). Six days of inanition (Days 14-20) again reduced ($P < 0.05$) cell size. After 7 to 10 consecutive days of inanition, cell size remained similar ($P > 0.05$) in fetuses from starved dams.

Figure 8. Day 20 fetal and placental weights from dams subjected to inanition as a percentage of fetal and placental weights of dams fed ad libitum throughout Days 0 to 20


DEVELOPMENT OF CONCEPTUSES AS PERCENTAGE OF FULL-FED CONTROLS
100
75
50
25
0
15-20
14-20
13-20
12-20
11-20
10-20
Alternate 0-20
DAYS OF GESTATION DAM SUBJECTED TO INANITION
FETUS
PLACENTA

Pregnant rats starved from Days 10 to 20 (10 days) lost a similar ($P > 0.05$) quantity of body weight as compared to nonpregnant rats of a similar ($P > 0.05$) body weight starved for 10 days (Figure 9). At autopsy carcasses of these pregnant rats subjected to 10 consecutive days of starvation had less ($P < 0.05$) dry matter ($33 \pm 0.6\%$ vs. $35 \pm 0.5\%$) and less ($P < 0.001$) lipid ($4.2 \pm 0.9\%$ vs. $11.3 \pm 0.7\%$) than that observed in rats fed ad libitum throughout gestation.

Table 9 presents the effects of inanition on the weight of the heart, kidneys, liver, gastrocnemius, and uterus. Inanition decreased ($P < 0.05$) heart weight in pregnant and nonpregnant animals, but the proportion of heart weight to body weight was greater ($P < 0.01$) in unmated rats whether they were starved or fed ad libitum. Pregnant rats subjected to inanition or fed ad libitum had similar ($P > 0.05$) proportions of heart to body weight. Starvation reduced ($P < 0.01$) kidney weight in pregnant and nonpregnant rats as compared with ad libitum-fed rats; however, the kidney weight per g of body weight was greater ($P < 0.01$) in starved, and in unmated animals. Inanition decreased ($P < 0.01$) liver weight and also reduced ($P < 0.01$) the quantity of liver per 100 g of body weight. Pregnant rats starved 5 or 10 days possessed a larger ($P < 0.01$) liver and more ($P < 0.01$) liver per 100 g of body weight than unmated rats starved 5 or 10 days. The gastrocnemius in unmated rats starved for 5 or 10 days remained similar ($P > 0.05$) to that found in ad libitum-fed unmated controls. Furthermore, the gastrocnemius in these unmated rats was similar ($P > 0.05$) to that in ad libitum-fed pregnant rats. Inanition reduced ($P < 0.01$) the Day 20 gastrocnemius weight in

Figure 9. Body weight change for pregnant (□) and nonpregnant (●) rats subjected to 10 days of inanition

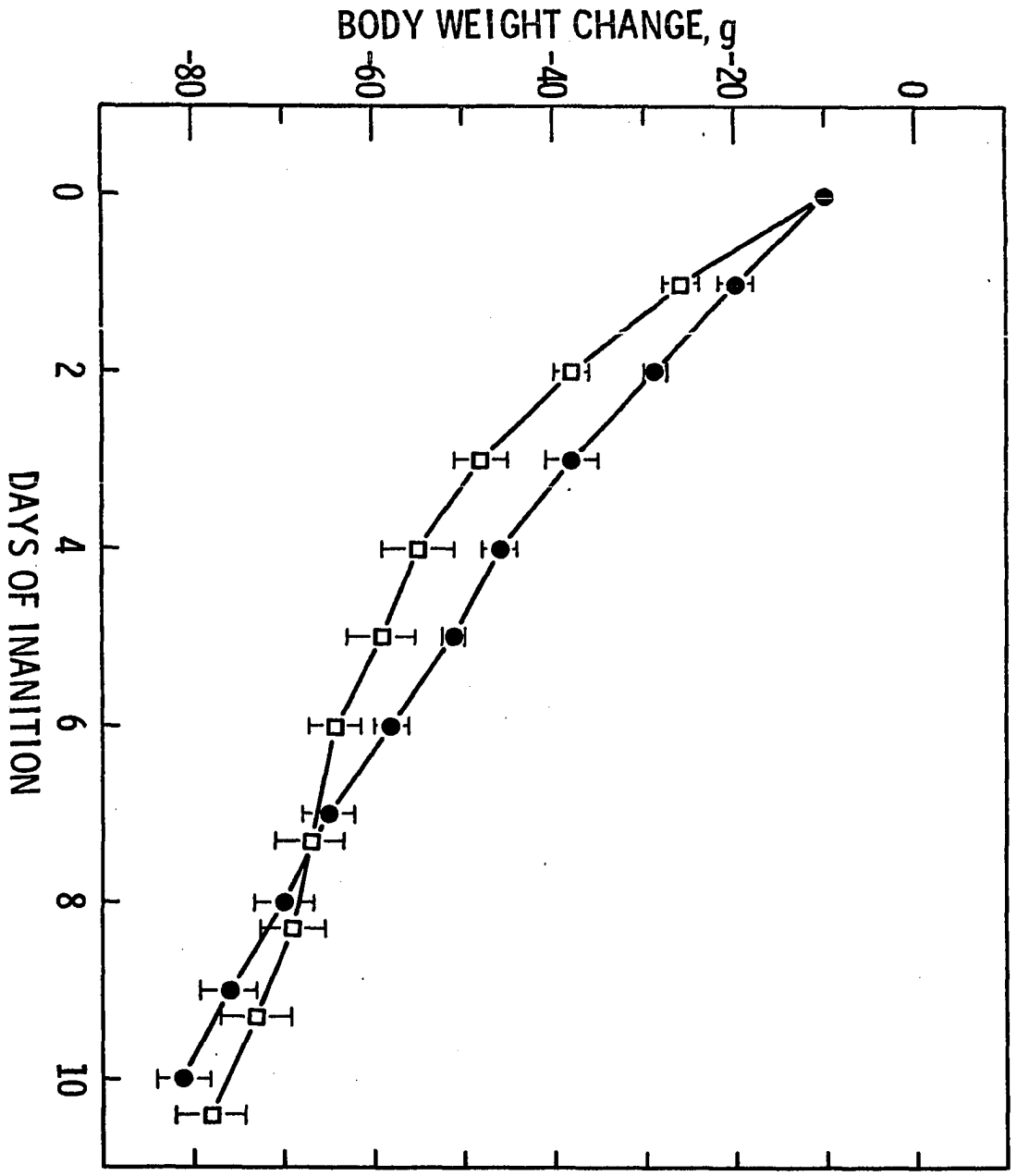

BODY WEIGHT CHANGE, g
-80
-60
-40
-20
0
DAYS OF INANITION
0
2
4
6
8
10

Table 9. Weight of the heart, kidneys, liver, gastrocnemius, and uterus from rats subjected to inanition and rats ad libitum-fed

Days of gestation dam subjected to inanition, days	Number days of inanition	Heart		Kidneys	
		wt., mg[a]	mg/g body wt.[a]	wt., g[a]	mg/g body wt.[a]
None	0	928 ± 52	2.8 ± 0.1	1.7 ± 0.1	5.0 ± 0.2
None[b]	0	869 ± 30	3.4 ± 0.1	1.9 ± 0.1	6.8 ± 0.1
15-20	5	767 ± 20	3.1 ± 0.1	1.4 ± 0.1	5.7 ± 0.1
0[b]	5	733 ± 33	3.7 ± 0.1	1.3 ± 0.1	6.6 ± 0.1
14-20	6	777 ± 25	3.1 ± 0.1	1.5 ± 0.1	6.1 ± 0.3
13-20	7	731 ± 23	3.2 ± 0.1	1.4 ± 0.1	6.1 ± 0.2
12-20	8	685 ± 14	3.1 ± 0.1	1.3 ± 0.1	5.8 ± 0.2
11-20	9	679 ± 38	3.1 ± 0.1	1.3 ± 0.5	5.7 ± 0.2
10-20	10	710 ± 18	3.0 ± 0.1	1.4 ± 0.1	5.9 ± 0.2
0[b]	10	771 ± 23	3.5 ± 0.1	1.4 ± 0.1	6.2 ± 0.1
Alternate 0-20	10	908 ± 30	2.9 ± 0.1	1.7 ± 0.1	5.1 ± 0.2

[a]Mean and S.E.

[b]Not mated.

Liver		Gastrocnemius		Uterus
wt., g[a]	g/100g Body wt.[a]	wt., g[a]	mg/g body wt.[a]	wt., g[a]
11.4 ± 0.4	3.4 ± 0.1	1.7 ± 0.1	5.0 ± 0.1	4.0 ± 0.2
9.3 ± 0.3	3.3 ± 0.1	1.9 ± 0.1	6.7 ± 0.2	0.40±0.05
7.5 ± 0.4	3.0 ± 0.1	1.2 ± 0.1	4.9 ± 0.1	3.2 ± 0.2
5.0 ± 0.2	2.3 ± 0.1	1.5 ± 0.1	7.1 ± 0.2	0.32± 0.06
7.7 ± 0.5	3.0 ± 0.2	1.2 ± 0.1	4.9 ± 0.1	3.3 ± 0.2
6.5 ± 0.4	2.8 ± 0.2	1.2 ± 0.1	5.0 ± 0.2	3.0 ± 0.1
6.3 ± 0.2	2.8 ± 0.1	1.2 ± 0.1	5.2 ± 0.1	2.8 ± 0.1
5.6 ± 0.3	2.6 ± 0.1	1.2 ± 0.1	5.3 ± 0.2	3.0 ± 0.1
6.5 ± 0.2	2.8 ± 0.1	1.3 ± 0.1	5.4 ± 0.1	3.1 ± 0.1
4.6 ± 0.2	2.0 ± 0.1	1.6 ± 0.1	7.0 ± 0.2	0.27± 0.03
11.0 ± 0.6	3.4 ± 0.4	1.6 ± 0.1	5.1 ± 0.2	4.2 ± 0.2

pregnant rats. Five consecutive days of inanition reduced ($P < 0.01$) the gastrocnemius weight in pregnant rats to 1.2 g. After 4 to 5 more days of inanition, the gastrocnemius in pregnant rats remained similar ($P < 0.05$). In proportion to body weight, unmated rats had a larger ($P < 0.01$) gastrocnemius compared with pregnant rats whether full-fed or subjected to inanition. In pregnant rats starved or fed ad libitum the gastrocnemius remained a similar ($P > 0.05$) proportion of the body weight. Inanition depressed ($P < 0.001$) uterine growth in pregnant rats (Table 9). Organ weights and organ weights on a unit of body weight basis of rats starved and fed every other day throughout gestation were similar ($P > 0.05$) to pregnant rats fed ad libitum throughout gestation (Table 9).

Ovarian weights in pregnant rats, starved 5, 6, 7, or 8 days were less ($P < 0.05$) than ovarian weights of ad libitum-fed pregnant rats or rats subjected to 9 or 10 consecutive days of inanition (Table 10). Fasted rats had similar ($P > 0.05$) or in some instances (6, 8, 9, 10 days of inanition) more ($P < 0.05$) ovarian weight per unit of body weight. Inanition for 5 to 10 consecutive days increased ($P < 0.05$) adrenal weight of pregnant rats as compared with ad libitum-fed pregnant animals. Unmated rats subjected to 5 or 10 days of inanition had an adrenals weight similar ($P > 0.05$) to full-fed unmated rats. In relation to body weight on Day 20, starved rats had an extremely high ($P < 0.001$) proportion of adrenal gland per 100 g of body weight (Table 10). As

Table 10. Weights of ovaries and adrenals from rats subjected to inanition and rats fed ad libitum

Days of gestation dam subjected to inanition	Number days of inanition	Ovaries wt[a]		Adrenals wt[a]	
		mg	mg/100 g body wt	mg	mg/100 g body wt
None	0	99 ± 4	30 ± 1.3	58 ± 3	17 ± 0.6
15-20	5	84 ± 3	34 ± 1.3	71 ± 3	28 ± 1.0
14-20	6	86 ± 5	35 ± 1.5	70 ± 8	32 ± 5.3
13-20	7	79 ± 4	34 ± 1.3	75 ± 8	32 ± 2.2
12-20	8	85 ± 3	38 ± 1.5	71 ± 3	32 ± 1.7
11-20	9	96 ± 5	44 ± 2.3	75 ± 5	35 ± 1.8
10-20	10	98 ± 2	41 ± 1.0	69 ± 3	30 ± 1.7
Alternate 0-20	10	106 ± 6	33 ± 2.1	62 ± 4	19 ± 1.2
0[b]	5	59 ± 4	28 ± 1.3	60 ± 3	28 ± 0.9
0[b]	10	59 ± 4	27 ± 1.9	61 ± 2	28 ± 0.9
None[c]	0	73 ± 7	23 ± 1.9	64 ± 4	22 ± 0.8

[a]Mean and S.E.

[b]Not mated but subjected to inanition and autopsied at end of 5 or 10 days of inanition.

[c]Full-fed, not mated, autopsied.

before, pregnant rats starved every other day, Days 0-20 were similar ($P > 0.05$) in all respects to full-fed pregnant animals (Table 10).

Inanition reduced ($P < 0.05$) total serum protein in pregnant rats starved Days 14-20, 13-20, and 12-20 as compared with ad libitum-fed

mated rats on Day 20 (Table 11). Urea nitrogen and α-amino nitrogen were lower ($P < 0.05$) in dams starved Days 15-20 as compared with ad-libitum fed dams on Day 20. In dams of longer inanition periods serum urea nitrogen and α-amino nitrogen levels remained similar ($P > 0.05$) to ad-libitum fed dams on Day 20. Dams subjected to inanition Days 15-20, 14-20, 13-20, and 12-20 had lower ($P < 0.05$) serum glucose levels. Dams subjected to inanition Days 11-20 and 10-20 had serum glucose levels similar ($P > 0.05$) to full-fed pregnant animals on Day 20. Free fatty acids were elevated ($P < 0.05$) in pregnant rats starved Days 15-20, 14-20, and 13-20. Free fatty acids in the serum of rats starved Days 12-20, 11-20, and 10-20 were similar ($P > 0.05$) to that observed in animals on ad libitum feeding (Table 11).

Serum levels of calcium, potassium, and sodium were similar ($P > 0.05$) to ad-libitum fed dams on Day 20 when dams were starved Days 15-20, 14-20, 13-20, 12-20, and 11-20. Rats subjected to inanition Days 10-20 had lower ($P < 0.05$) serum calcium and sodium and elevated ($P < 0.05$) serum potassium (Table 12).

Table 11. Serum protein, urea nitrogen, α-amino nitrogen, glucose and free-fatty acids on Day 20 of rats subjected to inanition during later half of pregnancy

	Days of gestation dam subjected to inanition						
	0	15-20	14-20	13-20	12-20	11-20	10-20
Total protein, g/dl[a]	7.2 ± 0.4	6.4 ± 0.6	5.9 ± 0.2	5.8 ± 0.3	6.0 ± 0.5	6.3 ± 0.2	5.4 ± 0.5
Urea nitrogen, mg/dl[a]	24.0 ± 1.3	18.1 ± 1.4	20.6 ± 2.7	25.8 ± 3.3	24.9 ± 2.3	30.3 ± 5.2	25.5 ± 3.3
α-amino nitrogen μM/ml[a]	8.3 ± 0.4	7.0 ± 0.3	7.3 ± 0.9	7.5 ± 0.7	7.2 ± 0.8	8.6 ± 0.7	8.2 ± 0.4
Glucose, mg/dl[a]	86 ± 5	42 ± 3	63 ± 8	63 ± 8	44 ± 5	74 ± 9	72 ± 12
Free fatty acids, μ eq/dl[a]	74 ± 6	114 ± 12	110 ± 13	103 ± 2	105 ± 16	102 ± 13	84 ± 11

[a]Mean and S.E.

Table 12. Serum calcium, potassium and sodium on Day 20 of rats subjected to inanition the last half of pregnancy

	Days of gestation dam subjected to inanition						
	0	15-20	14-20	13-20	12-20	11-20	10-20
Ca, mg/dl[a]	11.1 ± 0.4	10.2 ± 0.4	9.9 ± 0.5	11.1 ± 1.3	10.1 ± 0.5	10.0 ± 0.3	10.0 ± 0.2
K, mg/dl[a]	25.1 ± 0.5	26.2 ± 1.0	27.6 ± 1.7	24.0 ± 1.2	27.5 ± 1.7	26.9 ± 1.2	28.1 ± 1.2
Na, mg/dl[a]	315 ± 6	300 ± 11	298 ± 6	308 ± 14	315 ± 11	302 ± 12	292 ± 6

[a]Mean and S.E.

DISCUSSION OF EXPERIMENT II

In this investigation, rats subjected to prolonged starvation during the later half of gestation maintained pregnancies and sustained a normal number of viable fetuses to Day 20, similar to those in ad libitum-fed rats (Table 6). These pregnant rats were subjected to inanition beginning Day 10 or later. Placentae from these starved dams sustained fetal development and adequate endocrine function of luteal tissue in spite of severe maternal deprivation. Anderson et al. (1974) and Kinzey and Srebnik (1963) found that in rats fed protein-free diets pregnancies were maintained when exogenous progesterone and estrone were given only a few days (Days 5-9); thereafter the placentae contributed major luteotropic support for the remainder of gestation. Kinzey (1968) suggested that endocrine support by the placentae continued in spite of an absence of dietary protein.

Starved pregnant rats sustained the growth of a normal number of viable fetuses by mobilization and efficient utilization of maternal tissue components. These pregnant rats lost maternal body weight. Non-pregnant rats lost similar quantities of total body weight as compared to pregnant rats starved for 10 days (Figure 9); however, the weight of the uterus and conceptuses represent weight lost from the body of the starved pregnant dam. Pregnant rats starved 10 days lost approximately 102 g; this represented 42 g of uterus and conceptuses plus 80 g of observed weight loss. Total body lipid and weight of heart, kidneys, liver, and gastrocnemius decreased in these pregnant rats subjected to

prolonged inanition. These starved dams converted maternal tissue components to sustain not only themselves, but also provided for development of rapidly growing conceptuses. When rats were starved every other day throughout gestation, weight of heart, kidneys, liver, and gastrocnemius remained similar to full-fed controls. In these rats starved every other day there was little augmentation of maternal body weight because most weight gained during gestation was attributed to that in the uterus and conceptuses. Ad libitum-fed pregnant rats augmented maternal body weight by 32 g after deleting weight of uterus and conceptuses at Day 20.

The weight of adrenal glands in relation to body weight of ad libitum-fed pregnant rats in this investigation was less than that observed in ad libitum-fed unmated rats. Pregnant rats subjected to inanition displayed an extremely high proportion of adrenal weight to body weight because adrenal weight increased slightly and body weight decreased markedly during starvation. The adrenal gland weight in proportion to body weight decreased throughout gestation in ad libitum-fed rats (Souders and Morgan, 1957).

Fetal development in starved dams was limited 75% to 50% of that observed in ad libitum-fed dams as indicated by a significant reduction in fetal weight, total protein, and total DNA (Tables 7 and 8). In comparison with other experiments which involved either protein or calorie restriction throughout gestation, these dams subjected to starvation demonstrated exceptional mobilization and utilization of their stores of protein and

energy to sustain growth of the conceptuses during the last trimester. Rats fed protein-free diets throughout gestation produced fetuses approximately 50% smaller than those in dams fed diets containing adequate protein (Anderson et al. 1974; Endo et al. 1974; Morishige and Leathem, 1972,; Callard and Leathem, 1970), whereas rats starved during the later half of gestation in the present investigation also produced fetuses which weighed 50% of that found in ad libitum-fed dams. Rats fed a diet deficient only in zinc produced fetuses which weighed 70% of that found in controls (Apgar, 1975). Dams, in this investigation, starved for 5 to 7 days were able to produce fetuses which weighed at least 70% of that observed in ad libitum-fed dams (Figure 8).

Fetal and placental development were restricted in dams subjected to inanition; however, as the period of starvation increased, fetal growth was limited more severely than placental growth (Figure 8). In guinea pigs fed either low-protein or low-calorie diets, the fetuses were more limited than the placentae (Young and Widdowson, 1975). In the pig, placental development was more restricted than embryonic development by prolonged inanition during the first trimester (Anderson, 1975).

In spite of severe demands upon the dam by combined effects of pregnancy and starvation, the serum concentrations of sodium, potassium, calcium, protein, urea, α-amino nitrogen, glucose, and free fatty acids of starved pregnant rats were in most instances similar to ad libitum-fed pregnant dams on Day 20 (Tables 11 and 12). The marked decline in serum glucose after only five days of inanition was observed in another study

after a short period of fasting in late pregnancy (Herrera et al. 1969), whereas serum free fatty acids were elevated during this time according to Jones (1976) and Herrera et al. (1969). In this investigation, prolonged starvation of pregnant dams reduced total serum protein concentration. Protein-free diets fed to pregnant rats also reduced serum protein concentration (Fisher and Leathem, 1965).

During the third trimester the conceptuses of the rat increase more than 10-fold in weight. Rats subjected to starvation for 5 to 10 days during the third trimester converted maternal stores of protein and energy to sustain growth of 9 to 12 conceptuses. Although development of these conceptuses was retarded in the starved dams, the conceptuses represented about 18% of the dam's body weight by Day 20. Ad libitum-fed dams sustained growth of a similar number of conceptuses which also represented about 18% of her body weight by Day 20.

SUMMARY OF EXPERIMENT II

Forty-eight pregnant rats subjected to inanition, beginning Days 10, 11, 12, 13, 14, or 15 and continuing through Day 20, all maintained pregnancy and produced a normal number of viable fetuses. These starved dams lost from 91 to 46 g of maternal body weight. Their heart, kidneys, liver, and gastrocnemius were all reduced in weight. Serum concentrations of calcium, sodium, potassium, protein, urea, α-amino nitrogen, free fatty acids, and glucose of most starved pregnant rats remained similar to that observed in ad libitum-fed pregnant rats on Day 20. Therefore, pregnant rats subjected to inanition during the third trimester converted tissue components to substrates for rapidly growing conceptuses. Fetal and placental development was limited from 75% to 50% of that observed for ad libitum-fed dams. Fetuses from starved dams contained less protein and had smaller cells as indicated by the protein-DNA ratio. However, weight of the conceptuses in relation to the body weight of the dam was similar to that of conceptuses from ad libitum-fed dams.

PART II: DEVELOPMENT OF CONCEPTUSES DURING INANITION IN THE PIG

INTRODUCTION

Normally gestation represents an anabolic process since pregnant animals terminate pregnancy with an increase in body weight, more than can be accounted for in the weight of the uterus and conceptus or conceptuses (Hytten and Thompson, 1968; Newton, 1952). Maternal malnutrition during pregnancy results in growth retardation of the conceptus or conceptuses. Diets low in energy or protein fed to pregnant pigs reduced birth weight of piglets (Atinmo et al. 1974a and 1974b; Buitrago et al. 1974; Pond et al. 1969).

In an earlier investigation it was determined that few pigs remain pregnant when the inanition period exceeded 37 days (Anderson, 1975). As in the rat fed a protein-free diet (Nelson and Evans, 1954), exogenous progesterone and estrogen were found to maintain pregnancy in pigs during prolonged inanition (Anderson, 1975). Therefore, a group of pigs in this investigation were ovariectomized and given exogenous progesterone and estrogen during inanition.

This investigation describes the effects of inanition beginning 10 to 14 days before mating and continuing through Day 34 of pregnancy on embryo survival, embryo and placenta development, and maternal serum protein, albumin, urea, calcium, potassium, and sodium.

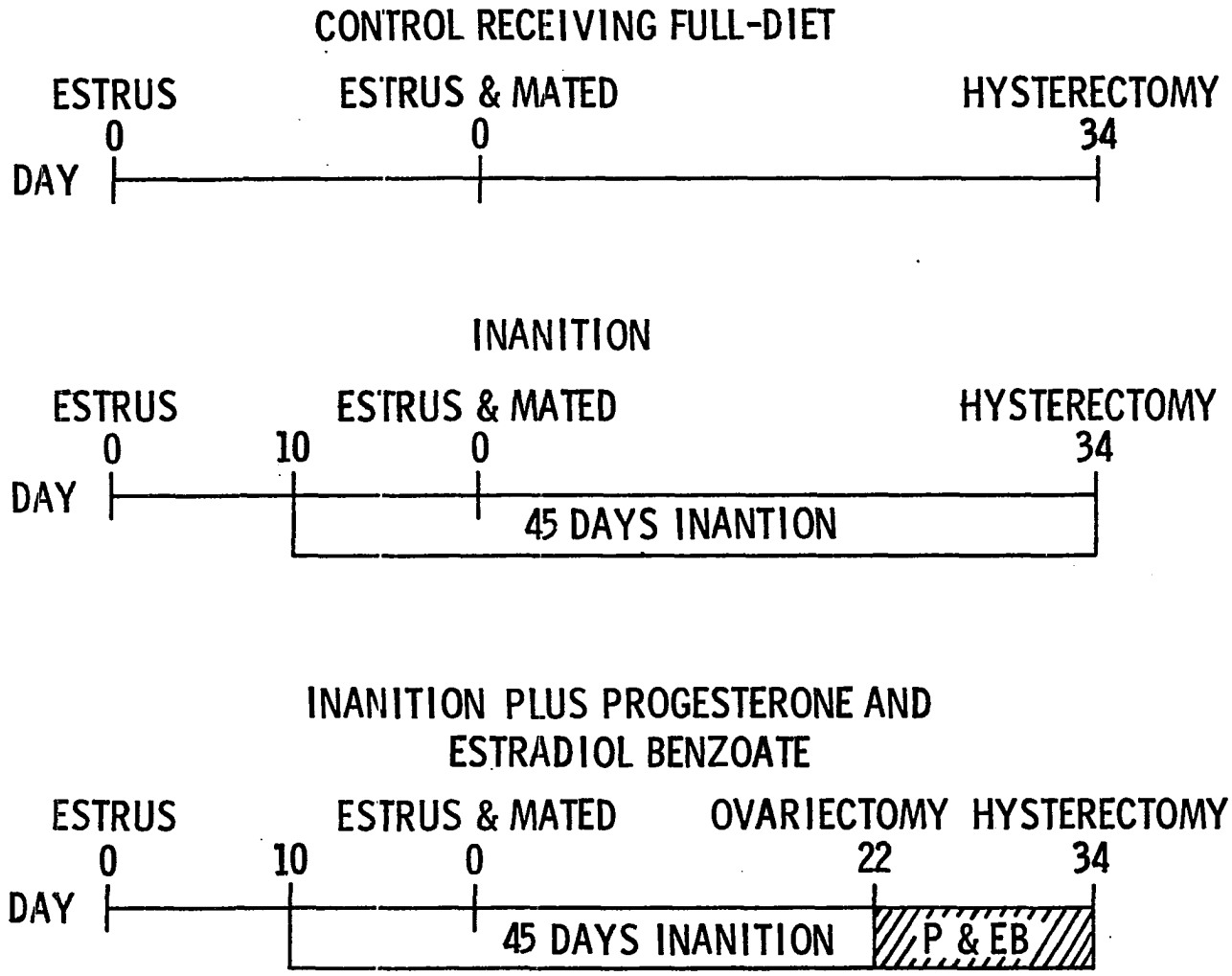
MATERIALS AND METHODS

Yorkshire gilts, about 120 kg body weight, were checked daily for estrous behavior with fertile boars, but were not allowed to mate. The first day of estrus was designated Day 0. There were 18 pigs in the experimental groups, and all of these pigs completed at least one normal estrous cycle (20 ± 2 days) before being assigned to a treatment group.

To insure that the experimental animals were free of communicable diseases that could reduce embryo survival and development or interfere with reproduction, the health of these animals was monitored by the Veterinary Medical Research Institute, Iowa State University, Ames. All experimental animals passed negative tests on serum samples for Brucellosis; Mycoplasma hyponeumoniae; Leptospira pomona, icterohemorrhagiae, canicola, hardijo, and grippotyphosa. Also, each animal tested negative for Bordetella bronchiseptica rhinitis.

At Day 10 after the second estrus, each gilt was maintained individually in a small pen (18-22°C). At this time, pigs were randomly assigned to one of three groups (six gilts per group): a) control, b) inanition, and c) inanition with progesterone and estradiol benzoate (Figure 10). Gilts were still checked daily for estrous behavior and were mated to fertile boars twice the next estrus. Control gilts were given a full diet (2.72 kg/day) of a 14% protein ration throughout the experimental period. Those gilts subjected to inanition were allowed access to water only. Inanition began on Day 10 after the second estrus (10-11 days before next expected estrus) and continued through mating to Day 34

Figure 10. Description of experimental groups indicating day of estrus, mating, and treatment periods


CONTROL RECEIVING FULL-DIET
ESTRUS
ESTRUS & MATED
HYSTERECTOMY
0
0
34
DAY
INANITION
ESTRUS
ESTRUS & MATED
HYSTERECTOMY
0
10
0
34
DAY
45 DAYS INANTION
INANITION PLUS PROGESTERONE AND
ESTRADIOL BENZOATE
ESTRUS
ESTRUS & MATED
OVARIECTOMY
HYSTERECTOMY
0
10
0
22
34
DAY
45 DAYS INANITION
P & EB

of pregnancy (Figure 10). Those pigs given exogenous progesterone and estradiol benzoate began inanition on Day 10 after the second estrus. However, at Day 22 after mating the ovaries of these animals were surgically removed. Inanition continued and progesterone (80 mg/100 kg body weight) and estradiol benzoate (500 µg/100 kg body weight) prepared as described by Anderson (1975) were injected daily from Day 22 to 34 after mating (Figure 10). Also, beginning Day 10 after the second estrus body weights were recorded every sixth day, and blood was collected every third day from all experimental animals via puncture of the cranial vena cava. Blood was allowed to clot at room temperature for 20-30 min., and then centrifuged in a refrigerated centrifuge at 3500 RPM for 20 min. Serum was removed and stored at -20°C until analysis.

On Day 34 after mating, hysterectomies were performed by a mid-ventral laparotomy with aseptic surgical techniques. Pigs were immobilized by intravenous injection of thiopental sodium (0.5-1.0 g, Abbott Laboratories) and maintained on a closed circuit system of halothane (1-6%, Ayerst Laboratories) and oxygen (600-1000 ml/min). Ovaries not previously removed (Day 22 after mating) were also taken at this time. All ovaries were weighed and the corpora lutea counted, dissected, and weighed. Immediately after removal, the uterus plus conceptuses was weighed and dissected. The position, condition (live-dead), crown-rump, and weight of each embryo was recorded. Placental tissue associated with each embryo was weighed. Also, the volume of placental fluid associated with each embryo was measured and recorded. After dissection,

the length and weight of each uterine horn were determined and recorded.

After hysterectomy, gilts subjected to inanition began a period of realimentation.

Maternal serum protein, albumin, and urea were determined by use of a Technicon autoanalyzer. Protein was measured by the biuret method (Technicon method file N-14b). Albumin was determined based on its dye-binding capacity with 2-(4'-hydroxyazo-benzene) benzoic acid (Rutstein et al. 1954) described in Technicon method file N-15b. Urea concentrations were measured by the reaction of urea and 2,3-butanedione-2-oxime in the presence of thiosemicarbazide (Marsh et al. 1965; Technicon method file N-1c).

Calcium, potassium, and sodium were determined by atomic absorption from 0.1 ml samples of maternal serum. To these 0.1 ml samples, 3.0 ml of 4% trichloroacetic acid containing 2500 ppm of strontium (strontium chloride) was added to precipitate serum proteins and overcome the depression of calcium absorption by phosphate. After centrifugation at 2500 RPM for 10 minutes, the supernatant was used to determine calcium and potassium by use of a Techtron atomic absorption spectrophotometer (Cary Instruments, Monrovia, California). The absorbance of calcium was determined at 4226.7 Å and the absorbance of potassium was determined at 7764.9 Å. A 25 µl sample of the supernatant was diluted with 4.0 ml of lithium diluent, 150 meq/l (lithium nitrate) and the absorbance at a wavelength of 5890.0 Å determined. Absorbance for known concentrations of calcium, potassium, and sodium were determined and graphed (absorbance against concentration). Unknown concentrations of sample absorbances

were determined from this graph.

Data were analyzed by computing Student's t based on a weighted average of sample variance.

RESULTS

Full-fed gilts exhibited estrous behavior and were mated at the expected time of 20 $\pm$ 2 days from their last estrus. Gilts subjected to inanition Day 10 of the estrous cycle before breeding exhibited estrus later than expected. These gilts showed estrous behavior and were mated 24 $\pm$ 0.7 days from their last estrus. This was different ($P < 0.01$) from their pretreatment estrous cycle of 20 $\pm$ 0.2, and different from the estrous cycle of full-fed gilts.

Initially, the body weight of all gilts was similar ($P > 0.05$). Those animals full-fed gained 9 kg while those subjected to inanition lost 31 to 27 kg of body weight over the 42 day period (Table 13). Gilts subjected to inanition and those gilts subjected to inanition plus progesterone and estradiol benzoate from Day 22 lost similar ($P > 0.05$) amounts of body weight. Overall, gilts subjected to inanition lost 28.6 kg body weight (0.68 kg/da) and full-fed gilts gained 9 kg body weight (0.21 kg/da; Figure 11). The uterus plus conceptuses of starved animals weighed less ($P < 0.05$) as compared to full-fed animals. Expressed as a percentage of the Day 34 body weight the uterus plus conceptuses was similar ($P > 0.05$) in starved and full-fed gilts (Table 1).

Six gilts were subjected to inanition only, beginning Day 10 of their second estrus. Thirty-four days after mating 4 of these gilts were pregnant. In those gilts subjected to inanition but ovaries removed on Day 22 postmating and given progesterone and estradiol benzoate, 6 of 6 maintained pregnancy to Day 34 postmating. All full-fed gilts were

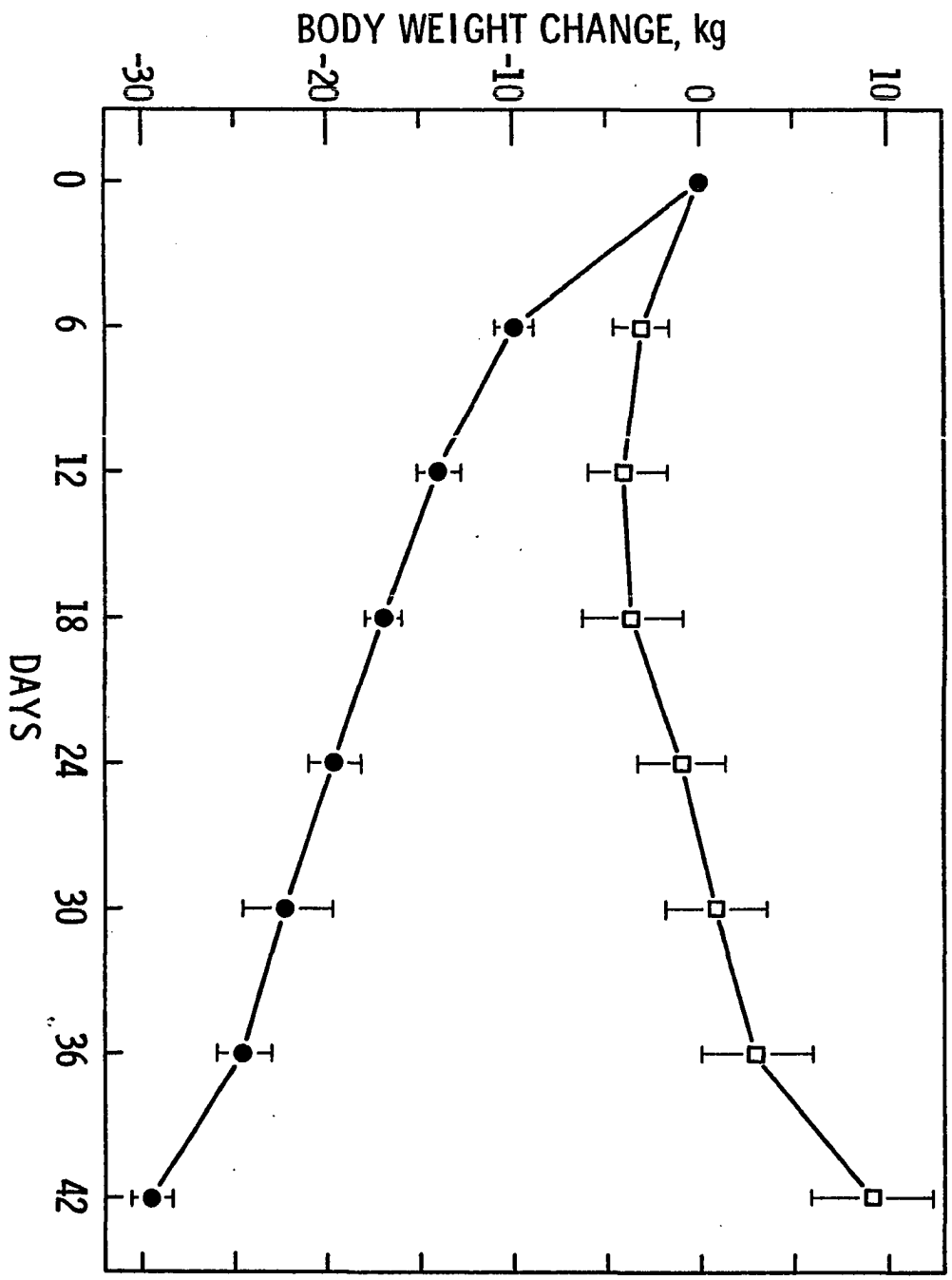
Table 13. Uterus plus conceptuses and body weight changes of pigs subjected to inanition 10 days before mating until Day 34 of pregnancy

Experimental group	Number of animals	Initial body wt, kg[a]	Body wt change by Day 34, kg[a]	Weight of uterus plus conceptuses on Day 34, kg	Uterus plus conceptuses as percentage of Day 34 body weight[a]
Inanition	4	132 ± 9	-31 ± 3	2.1 ± 0.2	2.1 ± 0.1
Inanition plus P and E_2B[b]	6	132 ± 6	-27 ± 1	2.6 ± 0.3	2.4 ± 0.2
Full-fed	6	129 ± 4	9 ± 3	3.1 ± 0.5	2.2 ± 0.6

[a]Mean and S.E.

[b]Inanition plus daily injections of progesterone and estradiol benzoate (from Day 22 after mating to Day 34).

Figure 11. Body weight change over 42 days for gilts full-fed (□) and for gilts subjected to inanition (●)


BODY WEIGHT CHANGE, kg
-30
-20
-10
0
10
DAYS
0
6
12
18
24
30
36
42

pregnant 34 days after mating.

Embryo weights on Day 34 were less ($P < 0.05$) in dams subjected to inanition as compared to full-fed dams. Those dams subjected to inanition plus exogenous progesterone and estradiol benzoate from Day 22 after mating had embryo weights similar ($P > 0.05$) to starved gilts and full-fed gilts on Day 34 (Table 14). Placental weight was limited ($P < 0.05$) in pigs subjected to inanition. However, the quantity of placenta on a unit basis of embryo was similar ($P > 0.05$) in gilts subjected to inanition and given exogenous progesterone and estradiol benzoate from Day 22 to that observed in gilts subjected to only inanition or gilts given a full diet. The total placental fluid associated with each embryo was reduced ($P < 0.05$) by inanition. Gilts starved and given progesterone and estrogen from Day 22 had a volume of total placental fluid associated with each embryo similar ($P > 0.05$) to that found in full-fed gilts, and different ($P < 0.05$) from that observed in gilts subjected to inanition without steroids.

All gilts had a similar ($P > 0.05$) number of embryos on Day 34 and embryonic survival rate was high and similar ($P > 0.05$) in gilts subjected to inanition and full-fed gilts (Table 14).

Inanition reduced ($P < 0.05$) ovarian and corpus luteum weight by Day 34 (Table 15). Uterine weight and uterine weight on a unit of weight basis was similar ($P > 0.05$) for gilts subjected to inanition, inanition plus progesterone and estradiol benzoate, and full-fed gilts (Table 15).

Serum calcium and potassium were similar ($P > 0.05$) throughout the 45 day period (10 days before mating to 34 days after mating) for starved

Table 14. Conceptuses from pigs subjected to inanition 10 days before mating and until Day 34 of pregnancy

Experimental group	Number animals	Embryo wt[a] g	Placenta wt[a] g	Placenta per g embryo[a]	Mean placental fluid vol, ml[a]	Number embryos[a] per gilt	Embryonic survival rate[a,b]
Inanition	4	2.1 ± 0.2	18.1 ± 1.5	10.2 ± 1.0	33 ± 4	11 ± 1	94 ± 4
Inanition plus P and E_2B[c]	6	2.7 ± 0.2	21.8 ± 2.4	8.2 ± 0.2	71 ± 6	12 ± 1	83 ± 4
Full-fed	6	3.0 ± 0.2	35.3 ± 5.8	10.5 ± 1.7	107 ± 18	12 ± 2	88 ± 10

[a]Mean and S.E.

[b]Number of embryos Day 34 divided by number of corpora lutea.

[c]Ovariectomy Day 22 of pregnancy then daily intramuscular injections of progesterone and estradiol benzoate through Day 34.

Table 15. Ovaries, corpus luteum, and uterus weight at Day 34 of gestation in pigs subjected to inanition 10 days before mating until Day 34 of pregnancy

Experimental group	Ovaries wt, g[a]	Mean corpus luteum wt, mg[a]	Uterus wt[a]	
			Total, g	g per cm
Inanition	9.4 ± 0.4	255 ± 20	1075 ± 28	4.2 ± 0.2
Inanition plus P and E_2B[b]	9.5 ± 0.5	252 ± 25	1188 ± 98	4.6 ± 0.3
Full-fed	11.8 ± 0.7	338 ± 22	1245 ± 111	5.3 ± 0.4

[a]Mean and S.E.

[b]Overiectomy Day 22 of pregnancy then daily intramuscular injections of progesterone and estradiol benzoate through Day 34.

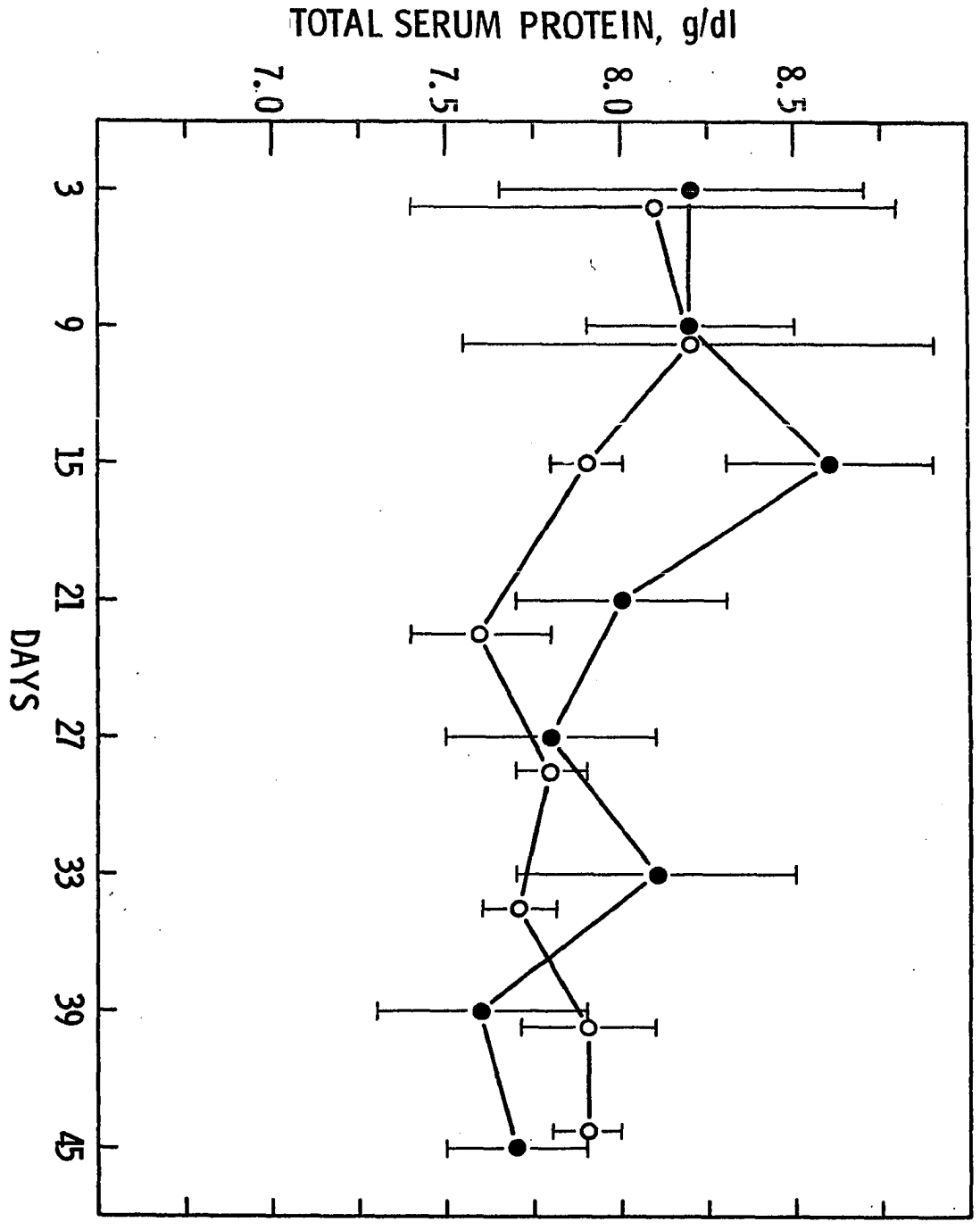
and full-fed gilts. Serum sodium was less ($P < 0.01$) in gilts subjected to inanition (Table 16). Serum protein in starved pigs was similar ($P > 0.05$) to full-fed pigs during the interval 10 days before mating to 34 days after mating (Figure 12), while serum urea and albumin were lower ($P < 0.01$) in pigs subjected to inanition (Figures 13 and 14).

Table 16. Serum calcium, potassium and sodium in gilts during 45 days of inanition or 45 days of full feeding

	Days							
	3	9	15	21	27	33	39	45
Calcium, mg/dl[a]								
Inanition	14.5 ± 0.8	13.4 ± 0.7	14.5 ± 1.0	13.0 ± 0.6	13.7 ± 1.0	14.3 ± 1.6	14.2 ± 0.4	14.4 ± 0.5
Full fed	13.3 ± 1.7	13.8 ± 1.3	11.9 ± 0.5	13.9 ± 0.9	11.8 ± 0.5	13.0 ± 0.6	12.8 ± 1.2	13.6 ± 1.0
Potassium, mg/dl[a]								
Inanition	20.8 ± 1.2	21.3 ± 1.4	19.7 ± 0.9	19.4 ± 0.5	20.1 ± 0.4	20.7 ± 0.3	19.5 ± 1.7	22.1 ± 1.3
Full fed	20.5 ± 0.6	20.7 ± 0.7	19.5 ± 1.0	20.1 ± 0.6	21.2 ± 0.9	21.6 ± 1.1	20.5 ± 0.7	19.1 ± 0.5
Sodium, mg/dl[a]								
Inanition	358 ± 11	352 ± 14	321 ± 20	314 ± 18	306 ± 15	295 ± 25	301 ± 33	308 ± 15
Full fed	370 ± 12	365 ± 13	345 ± 11	353 ± 13	375 ± 9	369 ± 10	377 ± 8	363 ± 13

[a]Mean and S.E.

Figure 12. Total serum protein over 45 days in pregnant gilts full-fed (o) and in pregnant gilts subjected to inanition (●)


TOTAL SERUM PROTEIN, g/dl
7.0
7.5
8.0
8.5
DAYS
3
9
15
21
27
33
39
45

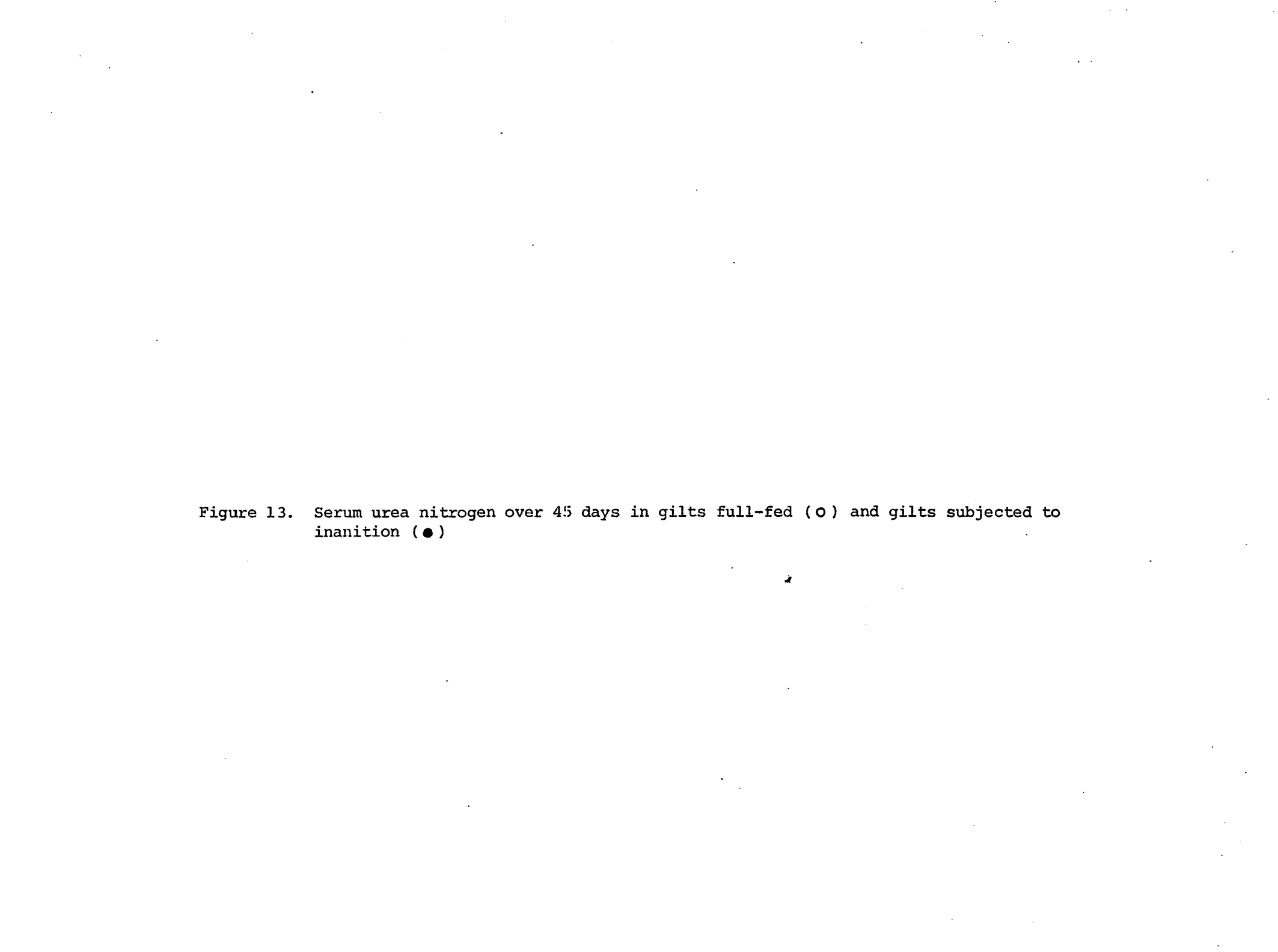


Figure 13. Serum urea nitrogen over 45 days in gilts full-fed (o) and gilts subjected to inanition (●)

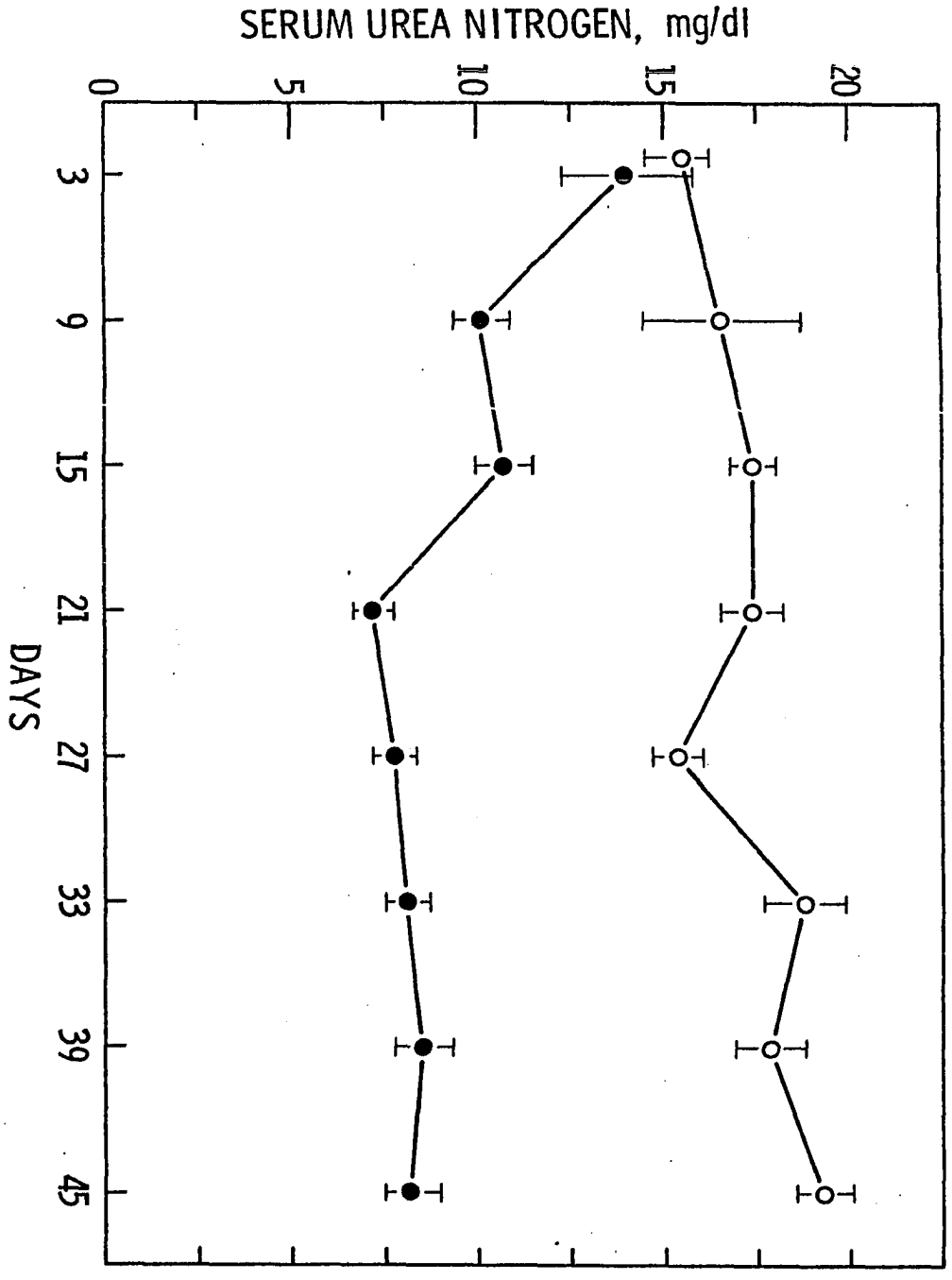
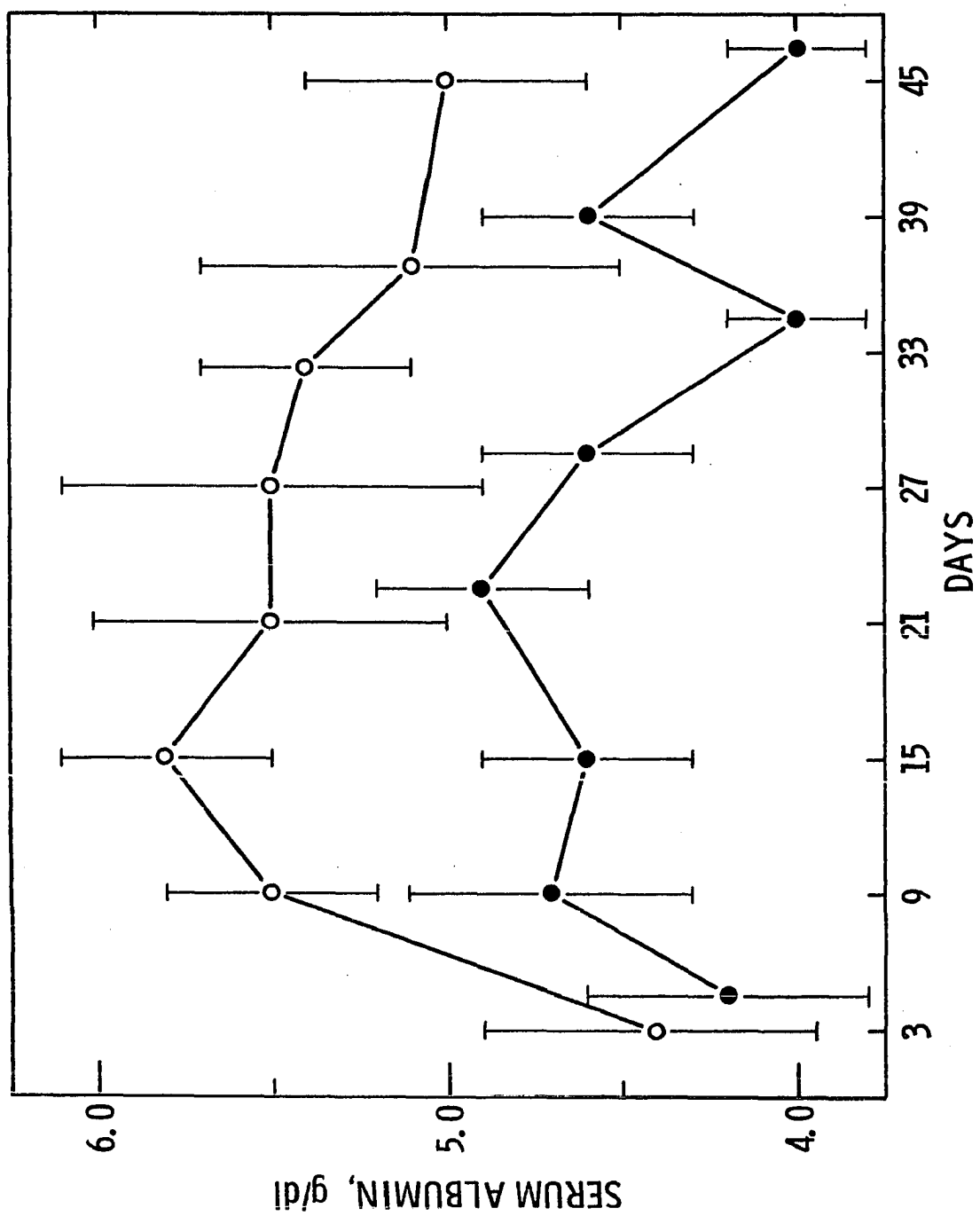

SERUM UREA NITROGEN, mg/dl
0
5
10
15
20
DAYS
3
9
15
21
27
33
39
45

Figure 14. Serum albumin over 45 days in gilts full-fed (○) and gilts subjected to inanition (●)


SERUM ALBUMIN, g/dl
6.0
5.0
4.0
3
9
15
21
27
33
39
45
DAYS

DISCUSSION

In this investigation, estrus was delayed in gilts subjected to inanition about 10 days before next expected estrus. In an earlier study involving pigs (Anderson, 1975) 37% of gilts subjected to inanition 10 days before next expected estrus failed to return to estrus. Severe underfeeding of guinea pigs delayed ovulation (Papanicolaou and Stockard, 1920). In the rat, starvation caused cessation of estrous cycles (Pomerantz and Mulinos, 1939).

Although these starved gilts exhibited delayed estrus, they mated and most were pregnant on Day 34 after mating. Of those animals subjected to inanition 4 of 6 remained pregnant to Day 34 and all starved animals (6 of 6) given exogenous progesterone and estradiol benzoate were pregnant Day 34. In a previous study only 1 of 6 gilts subjected to inanition maintained pregnancy to Day 34 (Anderson, 1975). Gilts in the present investigation were 12 to 15 kg heavier at the start of inanition compared with those of Anderson (1975); initial body weight may have some bearing on pregnancy maintenance.

Inanition of gilts limited embryonic and placental development. Embryos from starved gilts weighed 30% less than that of embryos from full-fed gilts while placentae of starved gilts were restricted approximately 50% less than normal. Thus, placentae were more restricted than the embryos by severe deprivation. Anderson (1975) also found that the placentae were more severely affected by severe deprivation of the dam than the embryos. In the rat and guinea-pig, the fetus is more severely

affected by malnutrition of the dam (Part I of this dissertation; Young and Widdowson, 1975).

Daily injections of progesterone and estradiol benzoate from Day 22 during inanition appeared partially effective in restoring weight limitation in conceptuses resulting from inanition. Values for mean embryo weight, mean placental weight, and the mean volume of total placental fluid with each embryo of those starved gilts injected with progesterone and estradiol benzoate were between the values of gilts subjected to inanition and full-fed gilts (Table 14). Anderson (1975) determined the nitrogen content of embryos and placentae and found that exogenous progesterone and estradiol benzoate restored the nitrogen content of embryos and placentae from starved dams to a level of that observed in full-fed dams. In rats, supplemental progesterone administered during pregnancy had no effect on fetal or placental weights (Bartholomeusz and Bruce, 1976).

Total protein in the serum of starved gilts was similar to that of gilts full-fed (Figure 12). Atinmo et al. (1974b) found serum protein in gilts to be lowered by feeding a protein restricted diet. Protein-free diets fed to pregnant rats also reduced serum protein (Fisher and Leathem, 1965). In this investigation, serum albumin was lower over the 45 day interval in gilts subjected to inanition (Figure 14). Atinmo et al. (1974b) observed low albumin levels in gilts receiving a protein restricted diet but not in those receiving an energy restricted diet during pregnancy. Serum urea in pregnant pigs was reduced by dietary protein restriction whereas that in dams on energy restriction remained

unaffected (Atinmo et al., 1974b). Starvation in this investigation reduced serum urea (Figure 13).

In spite of their different metabolic status, starved gilts were able to conceive and provide nutrients for the growth of conceptuses without affecting their survival during the first trimester.

SUMMARY

Gilts subjected to inanition approximately 10 days before next expected estrus exhibited delayed estrus. However, these gilts mated conceived, and had viable embryos on Day 34 after mating. Embryonic and placental growth was limited ($P < 0.01$) in gilts subjected to inanition. Restoration of embryonic and placental growth was observed in gilts maintained pregnant with daily injections of exogenous progesterone (80 mg/100 kg) and estradiol benzoate (500 µg/100 kg) during inanition. Whether pregnancy was maintained by endogenous hormones or exogenous progesterone and estradiol benzoate during inanition the embryonic survival rate was high (87%) and comparable to that observed in full-fed gilts (88%). Serum calcium, potassium, and protein concentrations in gilts subjected to starvation were similar ($P > 0.05$) to full-fed gilts. However, serum albumin, urea, and sodium were reduced ($P < 0.05$) in gilts subjected to inanition. Despite a body weight loss of approximately 30 kg, gilts converted their stores of protein and energy for growth of conceptuses during the first trimester.

GENERAL SUMMARY

Rats verified pregnant by laparotomy 6 days postmating (Day 6) were subjected to different intervals of inanition during gestation. All rats were fed ad libitum a commercially prepared diet before being subjected to starvation. Pregnant rats, in the first series, were maintained without feed beginning on Day 6 and continuing without feed 3, 4, 5, 6, 7, 8, or 9 days. The different periods of realimentation in these rats continued to Day 20, the day of autopsy. Inanition intervals of 4 (Days 6-10) or more days induced pregnancy failure in most (83%) rats. Daily injections of 5 mg of progesterone during the inanition interval of 4 days, Days 6-10, maintained pregnancy in all rats. Pregnant rats, in the second series, were maintained without feed beginning Days 10, 11, 12, 13, 14, or 15 and continuing through Day 20, the day of autopsy. All rats subjected to these periods of starvation maintained pregnancy. Embryonic survival rates remained high and similar in all rats pregnant on Day 20, regardless of whether they were ad libitum-fed or subjected to inanition beginning on Day 6, Days 10, 11, 12, 13, 14, or 15. Those few rats which maintained pregnancy during inanition intervals beginning Day 6 gained more or similar quantities of body weight as compared with those in which pregnancy failed, and provided for the growth of conceptuses during realimentation. Fetal and placental development, based upon weight and protein content, were limited only when realimentation of the dam extended into the third trimester. Although fetal and placental weight, protein, and DNA were limited in dams subjected to inanition beginning

Days 10, 11, 12, 13, 14, or 15, the proportion of conceptuses weight to Day 20 body weight of the starved dams was similar to that of ad libitum-fed dams. Serum concentrations of calcium, sodium, potassium, protein, urea, α-amino nitrogen, free fatty acids, and glucose of most starved pregnant rats remained similar to those observed in ad libitum-fed pregnant rats on Day 20. These pregnant rats starved during the last half of gestation lost more than 100 g body weight. Weight reductions of maternal heart, kidneys, liver, and gastrocnemius, and decreased total body lipid indicated utilization of maternal stores of protein and energy to sustain viable fetuses and provide for optimum fetal growth during conditions of severe deprivation.

Pigs subjected to inanition approximately 10 days before their next expected estrus exhibited delayed estrus. However, these pigs mated and sustained a normal number of viable embryos to Day 34 (45 days of inanition), as compared with full-fed gilts. Development of the conceptuses was limited in pigs subjected in inanition. Progesterone (80 mg/100 kg) and estradiol benzoate (500 μg/100 kg) injected daily from Days 22 through 34 maintained pregnancy in all pigs subjected to inanition. Serum concentrations of calcium, potassium, and protein during prolonged inanition remained similar to those observed in full-fed controls, while serum albumin and urea were reduced in those gilts subjected to inanition.

Despite severe nutrient deprivation imposed on the dam by inanition, the pregnant rat and pig sustained development of a high percentage of the potential young. Inanition induced pregnancy failure in

both the rat and pig in early pregnancy, however, exogenous progesterone supplied to the rat, and exogenous progesterone and estradiol benzoate, supplied to the pig, maintained pregnancy during extreme deprivation.

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APPENDIX

Table 17. Weight changes during realimentation for rats maintaining pregnancy and those in which pregnancy failed

Days of gestation dam subjected to inanition	Number days of inanition	Average weight change (g)[a]						
		Days 9-20	Days 10--20	Days 11-20	Days 12-20	Days 13-20	Days 14-20	Days 15-20
Pregnant[b]								
None	0	24 ± 2	21 ± 3	17 ± 2	12 ± 3	8 ± 3	3 ± 3	-5 ± 2
6-9	3	67 ± 2	39 ± 2	26 ± 2	22 ± 1	14 ± 2	8 ± 2	0 ± 2
6-10	4	-	73 ± 4	36 ± 4	27 ± 4	14 ± 5	7 ± 2	-1 ± 1
6-11	5	-	--	76 ± 8	45 ± 6	35 ± 7	23 ± 11	19 ± 12
6-12	6	-	--	-	79	46	30	29
6-13	7	-	--	-	-	72 ± 10	37 ± 8	19 ± 4
6-14	8	-	--	-	-	-	52 ± 5	26 ± 6
6-15	9	-	--	-	-	-	-	46 ± 6
6-10 + Prog[c]	4	-	74 ± 4	38 ± 5	30 ± 4	22 ± 3	13 ± 7	5 ± 2
Pregnancy failed								
None[d]	0	13 ± 2	14 ± 3	9 ± 2	12 ± 2	16 ± 3	11 ± 2	7 ± 1
6-9[e]	3	34 ± 3	15 ± 2	14 ± 2	8 ± 2	5 ± 1	4 ± 1	3 ± 1
6-10	4	-	47 ± 2	18 ± 2	13 ± 2	9 ± 2	9 ± 2	6 ± 1
6-11	5	-	--	54 ± 2	27 ± 2	22 ± 2	14 ± 2	10 ± 1
6-12	6	-	--	-	58 ± 4	34 ± 3	25 ± 3	19 ± 1
6-13	7	-	--	-	-	56 ± 2	32 ± 2	22 ± 2
6-14	8	-	--	-	-	-	59 ± 2	35 ± 2
6-15	9	-	--	-	-	-	-	59 ± 4

[a]Mean and S.E.

[b]Weight gain minus Day 20 uterus plus conceptuses weight.

[c]Progesterone 5 mg/da s.c. Days 5-10.

[d]Unmated.

[e]Sterile 3% NaCl injected into each implantation site on Day 9.

Table 18. Dry matter and lipid content of carcasses on Day 20 from *ad libitum* fed and starved dams

Experimental group	Carcass wet wt g[a]	Dry matter, %[a]	Lipid[a] g	Lipid[a] %
Ad libitum fed to Day 20	276 ± 12	35.2 ± 0.5	30.8 ± 1.6	11.3 ± 0.7
Starved Day 10-Day 20	169 ± 6	33.0 ± 0.6	7.1 ± 1.6	4.2 ± 0.9

[a]Mean and S.E.